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Screening of free fatty acids and sensorial changes of some *Panicum miliaceum* L. and *Avena sativa* L. varieties.

Diploma Thesis

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Declaration

I, Lenka Lošková, hereby declare that the whole of this diploma thesis is my own work unless otherwise referenced and acknowledged.

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Abstract

The effects of different storing conditions, three processing designs (flour, groats, whole grains) and content of unsaturated fatty acids on development of rancidity have been studied in seven proso millet (*Panicum miliaceum* L.) varieties. Storing changes were evaluated as titratable acidity; slightly modificated Czech State Norm (CSN) 56 0512-9 was used as a standard method. Evaluation preceded in two weeks intervals during 16 weeks. The most significant changes were found in processed grains, especially in groats. Great differences were found between storing conditions, titratable acidity almost doubled in laboratory conditions. The effect of long-term storage of whole grains on titratable acidity and sensory attributes in three oat (*Avena sativa* L.) varieties has been studied. Although titratable acidity of oat samples did not changed during 13 months in both storing conditions; sensory attributes significantly deteriorate in laboratory conditions. Significant differences were found among tested oat varieties. Significant correlation between titratable acidity and content of unsaturated acid was determined in proso millet and oats.

Keywords: Proso millet, oats, rancidity, free fatty acids, storing conditions, sensory evaluation

Abstrakt

U sedmi vybraných odrůdách prosa setého (*Panicum miliaceum* L.) byl studován vliv skladovacích podmínek, zpracovatelské varianty (mouka, šrot, celá zrna) a obsahu nenasycených mastných kyselin na proces žluknutí. Rozvoj žluknutí byl měřen podle České Státní Normy (CSN 56 0512-9) jako titrovatelná kyselost. Měření probíhalo ve dvoutýdenních intervalech po dobu 16 týdnů. Vyšší rozvoj žluknutí byl zaznamenán u zpracovaných zrn, zejména u šrotu. Žluknutí bylo významně ovlivněno skladovacími podmínkami, během skladování při pokojové teplotě se titrovatelná kyselost téměř zdvojnásobila. Vliv dlouhodobého skladování celých zrn na změny titrovatelné kyselosti a změny senzorických vlastností byl zkoumán na třech odrůdách ovsa. Ačkoli se titrovatelná kyselost nezměnila v žádném skladovacím prostředí, během skladování v pokojové teplotě došlo k významnému zhoršení senzorických vlastností. Titrovatelná kyselost i změny senzorických vlastností se výrazně lišily u jednotlivých odrůd. Rozvoj žluknutí byl ovlivněn obsahem nenasycených mastných kyselin u prosa i u ovsa.

Klíčová slova: proso, oves, žluknutí, skladování, mastné kyseliny, senzorické hodnocení

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List of used abbreviations

12:0	Lauric acid
14:0	Myristic acid
16:0	Palmitic acid
16:1	Palmitoleic acid
18:0	Stearic acid
18:1	Oleic acid
18:2	Linoleic acid
18:3	Linolenic acid
20:0	Arachic acid
20:1	Gadoleic acid
22:0	Behenic acid
22:1	Erucic acid
24:0	Lignoceric acid
AAPH	2,2'-azobis(2-amidinopropane) hydrochloride
Ala	Alanine
AOM	Active oxygen method
Arg	Arginine
Asp	Asparagic acid
A_w	Water activity
convar.	Convariety
CRI	Crop Research Institute, Prague
CSK	Former Czechoslovakia
CSN	Czech State Norm
cv.	Cultivar
CV	Coefficient of variation
Cys	Cysteine
FA	Fatty acid
FFA	Free fatty acid
FAO	Food and Agriculture Organization of the United Nations
Glu	Glutamic acid
Gly	Glycine
His	Histidine
HPLC	High performance liquid chromatography
Ile	Isoleucine
Leu	Leucine
LOX	Lipoxygenase oxidation
Lys	Lysine
Met	Methionine

NFE	Nitrogen free extracts
NMR	High-resolution nuclear magnetic resonance
NSL	Non-specific lipids
p-AnV	Anisidine Value
Phe	Phenylalanine
Pro	Proline
PV	Peroxide value
SE	Standard error
Ser	Serine
SUN	Former Soviet Union
TBA	Thiobarbituric acid value
Thr	Threonine
TNL	Total non-starch lipids
Tyr	Tyrosine
Try	Tryptophan
USA	United States of America
Val	Valine
var.	Variety
VUKROM	Agricultural Research Institute Kromeriz
WHO	World Health Organization
WSB	Water saturated *n*-butanol
WTS	Weight of thousand seeds

Content

Preface

Cereals are one of the most important crops for human consumption. They are the principal sources of energy, protein, vitamins, minerals and other important components. Cereals are of a special importance in developing countries, where they are usually the only source of essential nutrients and energy. Nevertheless, cereal grain is subject to quality loss during storage, often resulting in considerable diminution in their quality. Cereal grains with higher lipid content, high ratio of unsaturated fatty acids and high enzymatic activity are particularly sensitive to decreasing the quality (Galliard, 1983). Quality of grains and their final products can be controlled and maintained by suitable post-harvest and storage technology (Mills, 1996).

Proso millet is very suitable for tropic and subtropic regions; it is adapted to hot and semi-arid regions and grow well under low input technology and primitive techniques (Baker, 2004). Oats require humid and cooler conditions, but are well suitable for planting in tropics and subtropics at high altitudes (Gyeltshen, 2004). Both proso millet and oats are characterized by higher nutritional quality, digestibility, and high content of many beneficial nutrients (Butt *et al*., 2008; FAO, 1995). But their products are often related to very short shelf-life and they are characterized by development of impaired organoleptic properties.

The quality loss in stored grains is caused mainly by deterioration, a natural process which breaks down organic matter through physical, chemical or biological processes (Mills 1996). The chief form of adverse quality factors arising directly or indirectly from reactions of endogenous lipids (Heiniö *et al*. 2002) is referred to as rancidity (Galliard 1983). Lipid rancidity is evoked by two chemical processes. The first, hydrolytic rancidity is caused by lipase hydrolysis of triglycerides and leads to liberation of free fatty acids (Rossel, 1983). Concentration of free fatty acids, measured by titratable acidity, is reliable indicator of these processes (Smith, 2002; Shahidi and Wanasundara, 2002). Compounds responsible for negative flavour, e.g. ketones, aldehydes, alcohols, etc. (Grosch, 1987) are produced from reactions with oxygen (Deuel, 1957) in following oxidative rancidity.

Experiments with different plant materials and storage techniques are necessary for understanding of these processes. They will contribute to suitable storing of cereals and cereal products. Improvement of storage can significantly contribute to the household food security and nutrition of the inhabitants of developing countries. Surpass of adverse storage effects can support the interest of planting and consuming neglected crops.

1. Literature review

1.1. *Panicum miliaceum* L.

Panicum miliaceum, is known as proso millet, common millet, hog millet, broom-corn millet, Russian millet, prove millet, yellow hog, hershey, and white millet. *Panicum miliaceum* is a member of the tribe *Panicacea* of the *Poaceae*; it is generally included in millets. Proso millet is annual, short-day grass, adapted to a wide range of environmental conditions (Baltensperger, 1996). Proso millet is grown mainly for grains (Baker, 2004); it is of special value as staple food in many developing countries (FAO, 1995).

1.1.1. Millets

The "millets" do not mean a taxonomic group; millet is a collective term referring to a number of small-seeded annual grasses that are cultivated mainly as grain crops. The word "millet" comes from the word mil or thousand, pointing to the large number of grains that can be grown from a single seed. Millets are one of the oldest foods known to humans and possibly the first cereal grain used for domestic purposes (Baltensperger, 1996). Today millets rank as the sixth most important grain in the world. Millets are a significant part of the diet in northern China, Japan, Africa, India, and Egypt (Railay, 1999); they sustain about third of the world's population (FAO, 1995)

Millets include five genera, *Panicum, Setaria, Echinochloa, Pennisetum,* and *Paspalum. Paniceae*, the largest tribe of the *Poaceae*, includes economically important species: proso millet (*Panicum miliaceum* L.), foxtail millet (*Setaria italica* L. Beauv.), and pearl millet (*Pennisetum glaucum* R. Br.). Millets include approximately 6,000 varieties throughout the world.

Origin

Millets have been among the first of cultivated crops. The origin of millet is diverse, with varieties coming from both Africa (pearl millet, finger millet) and Asia (foxtail millet, proso millet). Millets have been grown there since prehistoric times (Magness *et al.*, 1971). Millets have been one of the staple foods in central and eastern Asia (mainly in China, India, and Russia), Europe and some parts of Africa during the very early ages (Oelke *et al*., 1990). In Africa, millets with sorghum have been the most important cereal up to the maize introduction (Murty and Kumar, 1995).

Economic importance

Although millets account for a little proportion of the world cereal production, for almost one-third of the world's population millets are a large part of the basic diet. In 2006, millets represented 1.4 % of the total world cereal production and occupied 4.9 % of the cereal production area. Statistical documentation of millet production can be incomplete, because statistics often combine data of millets, sorghum and other cereals in the category "coarse grain" (FAO, 1996). Millet production and proportion of production of individual millet species is demonstrated in Tables 1 and 2.

Table 1: Millet production (FAOStat, 2007).

Region	Total production [t]	Area [ha]	Yield [kg/ha]
World	31,780,872	32,845,741	967.58
Developing countries	30,695,098	31,875,704	962.96
Developed countries	1,085,774	970,037	1,119.31
India	10,100,000	9,500,000	1,063.00
Nigeria	7,705,000	4,970,100	1.549.00
Niger	3,200,000	5,200,000	615.00
China	1,820,900	900,500	2,220.00

Table 2: Relative importance (in %) of millet species in 1992-1994 (FAO, 1995).

Region	Pearl millet	Finger millet	Proso millet	Foxtail millet	Others
World	52	12	14	18	4
Developing countries	55	12	9	20	4
Developed countries	1	0	98	1	0

Millets have irreplaceable value in less-developed countries and in regions with inconvenient condition (Serna-Saldivar *et al*., 1991) (Figure 1); developing countries produce almost 97 % of world millet production. The greatest amount of millet was produced in India, Nigeria, Niger, China and Burkina Faso, representing 31.8 %, 24.2 %, 10.1 %, and 5.7 % of total millet production in 2006 respectively (FAOStat, 2007) (Table 1). In these countries, millets are produced directly for human consumption (Baltensperger, 1996); they are the main source of energy (FAO, 1995) and nutrients (Avallone *et al*., 2006; Tatala *et al*., 2007). In regions, where production of major food crops is not possible, millets ensure the food, nutritional and health security in many local communities (Ravi, 2004). The highest per capita consumption is in Africa, especially in the Sahel (FAO, 1995). Together with sorghums, millets represent a major source of protein for the population (Belton and Taylor, 2003). In developed countries, only limited quantities of millets was produced (3 %) in 2006. Among

developed countries, the Russian Federation, the United States and the Ukraine were the greatest producers in 2006 (FAOStat, 2007).

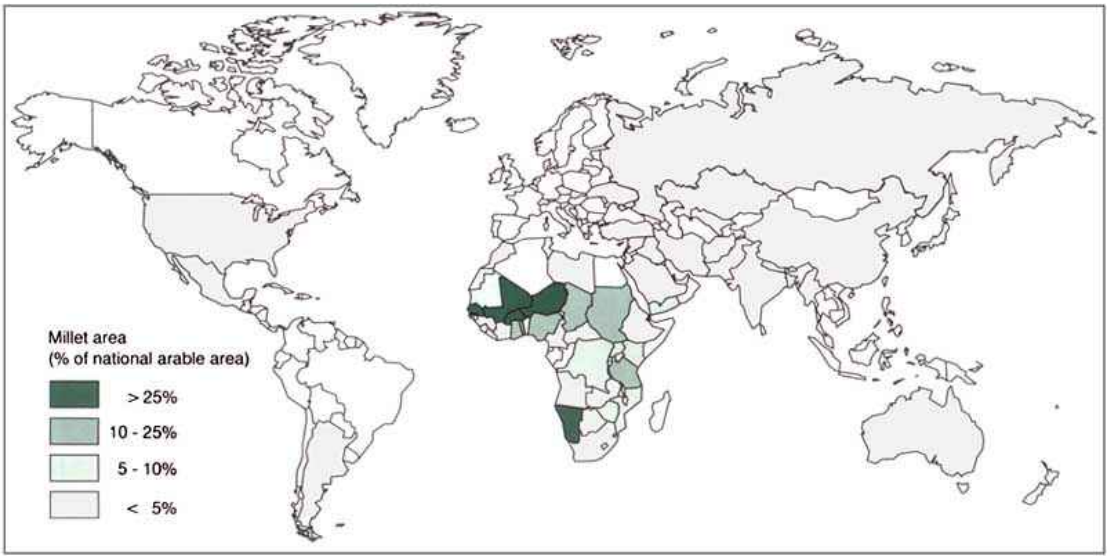


Figure 1: Relative importance of millets (FAO, 1996).

Although there are some trends to replace millets with more productive crops, millets due to some unique traits are irreplaceable in some regions. Fast maturing millets enable them to fit into more intensive cropping systems or avoid the unfavourable conditions. Millets tolerate a wide range of conditions including harsh environmental conditions. The seed costs are low; seeds can be stored for a long time without lost of viability. Millets have excellent nutritional properties, providing energy for a long time. There are a large number of ways of processing preparations; millets often require less cooking or preparation time (Dendy, 1995).

Millets are mainly produced by small-scale farmers as a subsistence crop for local consumption or localized trade (FAO, 1996) and they are usually traded only in small quantities. The absence of the large market outlets induces the fluctuation of prices (FAO, 1995). International trade of millet is estimated to range from 0.2 to 0.3 mil. t, i.e. approximately 1 % of total millet production (FAO, 1996). India, the United States and China are the major exporters. The major importer is the European community accounting for approximately half of total import (FAO, 2007).

Traditional uses

Besides grain production accounting the greatest amount of millet utilization (80 %) (FAO, 1996), millets are cultivated for grazing, green fodder, silage (together 7-10 %) (Rooney *et al*., 1996; FAO, 1995) or industrial processing (e.g. alcohol) (Agu, 1995;

Haggblade and Holzapfel, 2004), building materials, mulch and fuel (Lamers and Breuntrup, 1996; Nisanka and Misra, 1990; Varshney *et al*., 1987). Forage and feed utilization has been reviewed by Bramel-Cox *et al*. (1995).

Millets, produced mainly in small farms, are processed at home with traditional methods and techniques. Being coarse grain, it is very difficult to process millets grains, especially with primitive equipment. Millets are decorticated traditionally by women with a mortar and pestle, with stone mills (Murty and Kumar, 1995) or a hammer mill driven by diesel engine (FAO, 2007), followed by winnowing or washing (Serna-Saldivar *et al*., 1991). Millets are usually milled daily in necessary quantities (FAO, 2007).

The most common food prepared from millets and sorghums is porridge. Thick porridges, consumed in almost all regions cultivating millets, are very concentrated and it can be eaten with the hand. The procedure depends on a region; generally, flour (from decorticated, whole or fermented grains) is mixed with boiling water (FAO, 1995). Thin porridges are a mix of flour (usually composite flour) and water; milk and sugar is usually added (Murty and Kumar, 1995). Fermented porridges are result of the activity of microorganisms when the mix of flour and water is kept for few days (FAO, 1995).

Millet flour is used for wide range of steam-cooked products. In couscous, a steamed and granulated product, millet flour could replace wheat flour. Millet grains are very often used as a substitute when other raw material is not available or is expensive. Cracked grains and grits are cooked to make rice-like products (Murty and Kumar, 1995).

Malted grains are widely used to make alcoholic fermented beverages for centuries (Gadaga *et al*., 1999; Murty and Kumar, 1995); malt of pearl and finger millet is brewed to opaque beers (Agu, 1995).

Noodles have been a popular staple food in many parts of the world for at least 2,000 years (Lee *et al.*, 2007; Lu *et al*., 2005).

In India and Africa, fermented breads are extremely popular. Type of bread, way of the preparation and used type of millet depends on a region (Murty and Kumar, 1995). Injera (Ethiopia) and kisra (the Sudan) are the major fermented breads made from sorghum or millet flour. Unfermented dry pancakes are known by many local names, e.g. roti, chapatti, or tuwo (FAO, 1995). Nutritive value of some types of millet breads was evaluated by Badi *et al*. (1990), Al-Kanhal *et al*. (1990), and Sawaya *et al*. (1984).

1.1.2. Origin and distribution

Proso millet is one of the first cultivated cereal crops, probably prior to wheat (Baltensperger, 1996). It is thought to be first cultivated in China; the oldest evidence of its utilization in northwest China came from the Late Neolithic (Lee *et al*., 2007). Proso millet was spread via trade routes to India and Africa around 2,000 years ago (Serna-Saldivar *et al.*, 1991). It was known in Russia and the Middle East for thousand years (Baltensperger, 1996). Into Europe, proso millet was introduced around 1700 BC (Jacob *et al*., 2008), where its glumes was used in pottery manufacture in the Iron Age (Beranová, 1980). Proso millet was very important for Slavs; the word "proso" is an ancient Slav name. Proso millet was important during the Middle Ages, its importance dropped after introducing of potatoes and maize (Magness *et al*., 1971).

In recent times, proso millet is valuable as food and feed resources especially in semiarid regions of developing countries (Magness *et al*., 1971), or of economic importance as birdseed in developed countries (Serna-Saldivar *et al.*, 1991; FAO, 1996). In Asia, proso millet is important food crop due to its low requirements on conditions; it is extensively cultivated in India, China, Russia, in the Middle East including Iran, Iraq, Syria, and Turkey, and also in Afghanistan and Romania (Baltensperger, 1996). In Europe and also in Africa is of minor importance (FAO, 1996). In developed countries, proso millet is produced primarily for a high-value special market as bird seed (Serna-Saldivar *et al.*, 1991).

1.1.3. Uses

Proso millet utilization differs according to the region, as mentioned before. Utilization of proso millet is primarily for livestock feed, hay, or as an emergency catch crop or birdseeds (Baltensperger, 1996). Proso millet food products include groats and flour, which can be used in mixes with other flours or to produce biscuits (FAO, 1995).

Proso millet protein has some anti-atherogenic properties (Nishizawa *et al*., 1996); it lowers the plasma cholesterol (Nishizawa *et al*., 1990; Nishizawa, 2003), and has beneficial effect on liver injury (Nishizawa *et al*., 2002). Proso millet has some beneficial effects (heavier body weight, high egg production) on poultry (Luis, 1980). Linoleic acid isolated from proso millet has anti-tumour activity (Aburai *et al*., 2007).

1.1.4. Botanical characteristics

Proso millet has adventitious, shallow root system; the majority of the roots occur in the depth from 15 to 30 cm, maximum depth is about 120 cm (Blumenthal and Baltensperger,

2002). Brady and Tyler (1958) demonstrated production of hordenine, alkaloid with antibacterial and antibiotic properties, in proso millet shoots.

Erect and stout stem of proso millet reaches usually 30 to 90 cm (but can reached up to 130 cm) (Reddy *et al.*, 2007), which is covered with hairs in the lower and middle part. The upper part of the stem is filled with pulp. Stem consist usually of 5 to 7 internodes. Stem internodes elongate rapidly; the vegetative phase of the development can be completed in 16 to 20 days (Baltensperger, 1996). Proso millet produces from 1 to 5 tillers; the tillering is intravaginal (Moudrý *et al*., 2005).

Lanceolate leaves are 20-60 cm long and 1-3 cm wide with noticeable central vein. Leaf blades are slightly hairy on both surfaces and the edges; leaf sheaths are densely haired. Short ligules consist of a line of dense hairs; there are no auricles (Moudrý *et al*., 2005).

The inflorescence is branched panicle, 14 to 30 cm long (Reddy *et al*., 2007). Panicle can comprised 10-40 branches (Moudrý *et al.*, 2005). It takes 20 to 25 day from panicle differentiation to flowering, and 20 to 30 days to physiological maturity; the maturity proceeds from top to bottom of panicle (Baltensperger, 1996). The panicle shapes determine three varieties: *P. miliaceum* convar. *effusum* Alefeld (diffused), *P. miliaceum* convar. *contratum* Alefeld (with arched branches) and *P. miliaceum* convar. *compactum* Koernicke (compact) (Figure 2).

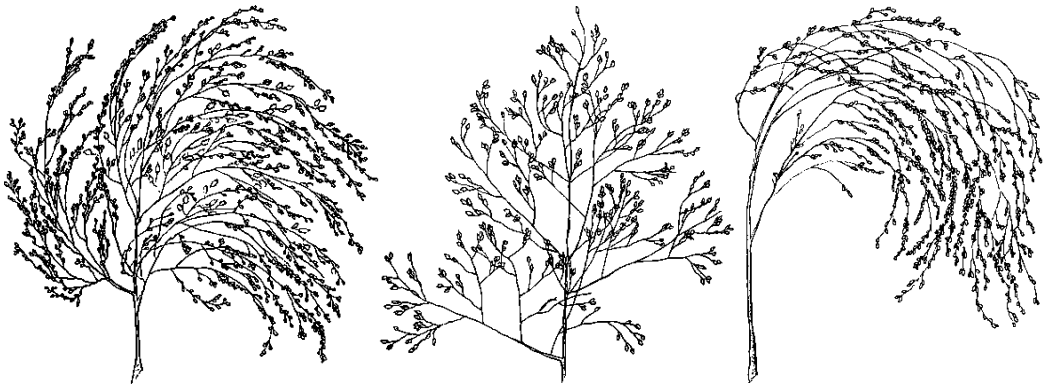


Figure 2: Panicles of proso millet (compact, diffused, and arched) (IBPGR, 1985).

The spikelets are borne singly at the ends of the branches. Each spikelet is about 5 mm long and consist of a fertile floret and a sterile floret, both enclosed between the outer glumes (Moudrý *et al*., 2005). The floret consists of the lemma and palea, enclosing three stamens and two stigmas (Zelený, 2005).The flower is opened by two lodicules (Moudrý *et al*., 2005).

Proso millet (2n = 36) is a self-pollinated crop, but natural cross-pollination may exceed up to 10 % (Baltensperger, 1996).

Proso millet grain characteristics

Millets demonstrate two types of grains (a) utricle type, where the pericarp is attached to the endosperm at one point (proso millet, finger millet, foxtail millet); and (b) caryopsis type, in which the pericarp is completely fused to the endosperm (sorghum and pearl millet) (FAO, 1995).

Proso millet has very small (about 3 mm long and 2 mm wide, Baltensperger, 1996) globular or broad-elliptical lustrous grain (Figure 3). The 1 000 kernel weight is about 5 g (varying between 4 and 8 g) (FAO, 1995). Seeds range in colour from white or cream to yellow, brown or nearly black (Baltensperger, 1996). The most common are brownish and yellow grains; red (Reddy *et al.*, 2007). Brighter-seeded varieties are preferred in special breakfast cereals (McDonald *et al*., 2000). Following redish-seeded ones are preferred in bird-seeded mix (Serna-Saldivar *et al*., 1991).

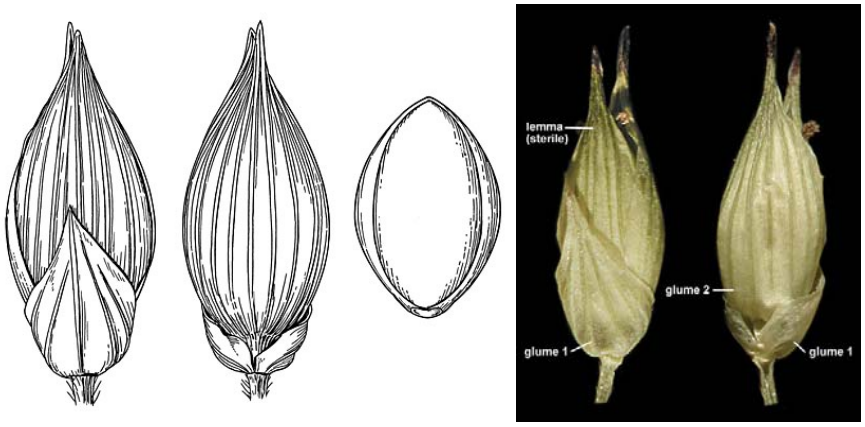


Figure 3: Grains of proso millet (Gardner, 2006).

Embryo of proso millet is very small, 1 100 × 310 µm (Lorenz and Kulp, 1991), but occupies about 5 % of the grain weight (Evers and Millar, 2001); the endosperm to embryo ratio is 11:1 to 12:1 (FAO, 1995).

Endosperm of proso millet accounts for about 70-75 % (Moudrý *et al*., 2005). The proportions of floury and corneous endosperm determine the texture of millet grain: (a) soft-textured (more floury endosperm) in pearl millet and sorghum, (b) hard-textured in foxtail millet; and (c) intermediate texture grains (endosperm evenly divided) in finger and proso millet (FAO, 1995).

The thickness of the testa is various, i.e. 0.2-0.4 mm, and comprised of only a single layer (FAO, 1995).

1.1.5. Ecology

Proso millet can be grown successfully on many soil and climatic conditions (Baltensperger, 1996). It is very adaptable; it can be grown in areas, where other crops have failed. Proso millet is short growing crop (mature in 60-90 days), it has low water requirement, it is heat and drought tolerable, and it grows well on poor soils and under primitive agricultural practices (Berglund, 1998; Baltensperger 1996). Thus, proso millet is of potential value in semiarid regions and developing countries.

Proso millet can be grown in a wide range of environmental conditions; it prospers better in hot, semi-arid regions. The optimum temperature for germination is between 35°C and 40°C (Oelke *et al*., 1990); suitable temperature ranges from 10°C to 45°C (Baltensperger, 1996). Proso millet can tolerate cold better than other C4 crop, but is sensitive to frost, so in mild zone is grown only during summer (Berglund, 1998).

Proso millet has possibly the lowest water requirement of cereal (mean annual precipitation as low as 300 mm) (FAO, 1996). Being C4, it has very low transpiration ration. The low straw grain ratio of proso millet contributes to its water use efficiency and adaptation to moisture-limited areas. Proso millet converts water very efficiently to dry matter/grain (Baltensperger, 1996) and provides stable yield of both grain and biomass under water stress (Emendack, 2005). It is one of the most efficient crops at removing moisture from the topsoil and converting it to dry matter (Berglund, 1998). Its drought-resistance is connected with avoiding the most unfavourable condition by short growing season (Oelke *et al*., 1990).

Due to its shallow root system, proso millet does not tolerate water-logging (Baltensperger, 1996); it grown well on well-drained loamy soils (Oelke *et al*., 1990). Proso millet has a low response to high-input conditions, because it is adapted to low fertility conditions and primitive practices (Baltensperger, 1996).

1.1.6. Agronomy

Millets are generally extensively planted on less fertile soils, under inconvenient conditions and with primitive techniques (Baker, 2004). Short-season proso millet is often planted as an emergency crop, when the other crop has poor germination or has been destroyed. Proso millet can follow in the spring after harvesting the winter crop (usually

winter wheat) (Baltensperger, 1996). Proso millet is more often grown in no-till than conventional system (Anderson, 1990; McDonald *et al*., 2000).

Proso millet is seeded in spring at average depth of about 2-3 cm; seedlings survive maximum depth of 7.5 to 13.5 cm. It requires higher temperature during germination. Seeding rate varies from 15-18 kg to 20-25 kg (Petr and Hradecká, 1997); higher seeding rate can establish a dense stand for preventing weeds (Oelke *et al*., 1990). The influence of seeding spacing was studied by Nelson (1977, 1981).

All millets respond to nitrogen and phosphorus fertilizers (Baker, 2004). Nitrate requirements are higher for proso millets planted following another crop under continuous cropping. Among common nitrate fertilizers, urea is the most susceptible (Blumenthal and Baltensperger, 2002). Fertilizing with sulphur or other micronutrients didn't show reliable response, as demonstrated by Blumenthal and Baltensperger (2002) in experiments in Nebraska. Proso millet requires a pH of 5.6 or higher (Oelke *et al*., 1990); with pH above 7.8, symptoms of iron chlorosis can occur (Baltensperger, 1996).

Millets compete against weeds with difficulty, especially at the seedling stage in the first two weeks (Baltensperger, 1996; Berglund, 1998). The most troublesome weeds are summer annual broadleaf weeds, annual and perennial grasses (Baker, 2004). Methods for controlling proso millets weeds include pre-plant tillage, weed-free seeds (Baker. 2004), high plant population, adapted variety selection, rotating proso millet with other crops (McDonald *et al*., 2000), and timely application of some herbicides (Anderson and Greb, 1987).

Diseases in millets are not common; proso millet is eminently free from disease resulting in economic losses (Baker, 2004). The major problems of proso millet are caused by bacterial stripes, head smuts, and kernel smut (Oelke *et al*., 1990). Smuts can be reduced with quality seed purchases, crop rotation and fungicide treatments; bacterial blights are usually controlled by copper fungicides (Baker, 2004).

Millets can be seriously affected by pests; the principal problems in proso millet are caused by thrips, armyworms, spider mites, corn borer, and grasshoppers (Berglund, 1998). All of them are traditionally controlled by insecticides (Oelke *et al*., 1990).

Determination of the most proper time of harvest is problematic as a result of diverse ripening. Proso millet is harvested when seeds in the upper half of the panicle are mature (Oelke *et al*., 1990). At this stage, leaves can be still green, but seeds in the lower part of the

panicle should have lost their green tinge (Berglund, 1998). At maturity, the grain of proso millet usually comprises about 20 % or less moisture (Baltensperger, 1996).

1.1.7. Proso millet grain composition

Like other cereals, proso millet grain is predominantly starchy. Proso millet is highly nutritious, one of the least allergenic and easily to digest. It contains many beneficial nutrient – it is rich in vitamins, especially in B-complex and vitamin E; it is a great source of minerals, iron, magnesium, phosphorous and potassium (Serna-Saldivar and Rooney, 1995). High fibre content is connected with beneficial influence on health (FAO, 1995). Proso millet composition and its comparison with other millets are demonstrated in Tables 3 and 4, where the mean value and range of values are presented. This way of expression of values is employed subsequently.

Table 3: Comparison of millet grains composition (FAO, 1995).

Millet	Protein	EE	Crude Fibre	Ash	NFE	Energy [kcal]
	[g in dry matter]					
Proso	11.6 11.3-12.7	4.2 3.8-4.9	12.0 7.9-19.2	3.6 3.1-4.2	70.4 66.3-73.4	364
Pearl	14.5 6.9-20.9	5.3 3.3-6.9	2.2 0.9-3.6	2.1 0.3-5.1	72.5 59.8-80.6	363
Japanese	11.6 11.2-11.8	5.8 5.5-6.3	14.7 11.6-16.3	4.7 3.0-5.6	63.2 60.9-67.8	300
Foxtail	13.0 12.6-14.0	4.8 4.6-5.0	10.0 9.3-10.6	4.0 3.7-4.3	68.2 66.1-69.7	351
Finger	8.0 4.9-11.3	2.0 0.9-7.7	3.6 0.7-8.0	3.0 2.0-5.0	72.4 69.3-73.4	336

EE = Ether extracts
NFE = Nitrogen Free extracts

Table 4: Composition of proso millet grain, various authors.

	Protein	EE	NFE	Ash	Fibre
	[% in dry matter]				
FAO, 1995	11.60	4.20	70.00	3.60	5.20
Oelke *et al*., 1990	12.00	4.00	75.00	-	8.00
FAO, 2007	13.71	1.76	72.26	1.07	0.10
Serna-Saldivar and Rooney, 1995[c]	13.40	6.70	69.40	4.20	6.30

Lipids

The lipids are chemically heterogeneous and subject to too great variation in composition, that there is no widely-accepted definition for them and any strict classification (Deuel, 1957). The most common lipid classification is based on physical properties at room

temperature (liquid/solid), the polarity (polar/neutral) and structure (straight/branched). Although lipids are relatively minor constituents in cereal grains, they are irreplaceable in nutritional value and functional properties of the grain. They can be found as intracellular components in cereal grain principally in spherosomes, protein bodies and starch granules (Fujino *et al*., 1996).

Proso millet contains from 1.8 to 6.7 % lipids, and about 24 % of the grain lipid can be found in the embryo (FAO, 1995). Major cereal grain lipids are acyl lipids which consist of free fatty acids (FFA) and fatty acids (FA) esterified to alcohols, primarily glycerol and its derivatives; the most abundant part are triglycerides (Fujino *et al*., 1996) (Table 5).

Table 5: Lipid composition in proso millet grain (Lorenz and Kulp, 1991).

Lipid class		Lipid composition (% lipids)				
		Free	Bound	Total NSL		Total
				C-M	WSB	
Nonpolar lipids	Steryl esters + Hydrocarbons	8.7	12.1	6.0	4.8	3.0
	FFA	3.1	9.1	16.7	14.5	1.8
	Sterol	4.5	11.4	9.5	5.4	5.4
	Monoglycerides	15.2	33.4	3.3	3.5	2.8
	Diglycerides	2.3	4.3	6.9	7.5	2.8
	Triglycerides	66.4	34.7	44.7	38.6	73.5
Polar lipids		15.2	33.4	13.1	25.9	14.3

NSL = non-specific lipids
TNL = total nonstarch lipids
WSB = water-saturated *n*-butanol
C-M = $CHCl_3$-CH_3OH

Cereal grains are very rich in unsaturated fatty acids (Fujino *et al*., 1996). The fatty acid profile of proso millet showed that saturated fatty acids occupy about 20 % while unsaturated fatty acids totalled 78 to 82 % (FAO, 1995). The most common saturated fatty acids are palmitic (16:0) and stearic (18:0) acids, whereas the chief unsaturated fatty acids are oleic (18:1), linoleic (18:2) linolenic (18:3) and palmitoleic (16:1) acids (Fujino *et al*., 1996) (Table 6). During the storage or processing of cereals, decomposition of fatty acids may produce low-molecular weight (aldehydes, ketones and others); these products cause a change of flavour and quality. These processes are described in chapter Rancidity.

Table 6: Fatty acid composition in proso millet grain, various authors.

	FA [%]					
	16:0	**16:1**	**18:0**	**18:1**	**18:2**	**18:3**
Lorenz and Hwang,1986	8.3 7.2-10.0	0.2 0.1-0.3	2.0 1.5-3.4	20.8 18.1-24.2	65.8 62.2-69.5	1.7 1.3-2.2
Serna-Saldivar and Rooney, 1995	11.3			21.4	64.9	2.5

Carbohydrates

Carbohydrates are quantitatively the major components of grains. The high molecular weight polysaccharides, comprise reserve polysaccharides and the structural polysaccharides, account for approximately 60-70 % of weight of the whole proso millet grain. The low molecular weight carbohydrates are up to 3 % of weight (Stone, 1996).

Starch is the major storage form of high molecular carbohydrates; proso millet contains about 60 % of starch (Stone, 1996) (Table 7). Starch is organized to granules with concentric ring structures; proso millet starch granules are smaller in comparison to other millets (Kumari and Thayumanavan, 1998). Table 8 summarizes proso millet starch properties. Starch is a highly organized association of two polysaccharides, amylopectin and amylase. Proso millet starch is compound on average of 73 % of amylopectin and 24 % of amylase. Proso millet has been reported to have also glutenous or waxy varieties with minute mount of amylase, i.e. 1.3 to 5.1 % (Graybosch and Baltensperger, 2004).

Table 7: Comparison of starch content (FAO, 1995).

Millet	**Starch**	**Amylose**	**Pentosan**	**Glucans**
	[%]			
Proso	60	24	0.17	0.21
Foxtail	47	18	5.5	-
Finger	57-59	6-18	6.2-7.2	-
Pearl	56-65	17-22	2-3	-

Table 8: Starch properties of proso millet (Lorenz and Kulp, 1991).

Amylose [%]	**Gelatinization**		**Water-binding capacity [%]**	**Swelling at 90°C**	**Solubility at 90°C**
	Initial [°C]	**Final [°C]**			
28.2	56.1	61.2	108.0	12.0	6.89

Cereal structural polysaccharides, such as cellulose, hemicelluloses, pectins, oligosaccharides, gums and various lignified compounds are generally define as dietary fibre. Cereals are a rich source of fibre; total dietary fibre in cereal grain varies from 8 % to 20 % (FAO, 1995). Fibre can be divided into water soluble and insoluble category; majority of fibre

is insoluble (Serna-Saldivar and Rooney, 1995). Insoluble fibre has structural and protective functions; it may have beneficial influence in diseases of the gastrointestinal tract. While soluble fibre components may help lower elevated blood cholesterol and sugar levels (FAO, 1995). Proso millet contains about 6.5 % of soluble fibre (Ravi, 2004).

The amount of the low molecular weight carbohydrates in the grain decreases as the grain matures and at maturity reaches up to about 3 % of its weight, the highest concentrations are found in the tissues of the embryo (Stone, 1996). The most prominent low molecular weight carbohydrates of proso millet grains are a non-reducing disaccharide, sucrose, followed by raffinose; glucose, fructose, and galactose are presented only in minute amounts (Becker and Lorenz, 1978).

Proteins

Proteins are the second major component in all cereal grains. They have major effects on quality, whether for food, feed or processing. The quality of protein is crucial important in regions where human diets consist chiefly of cereals (FAO, 1995), proteins of cereal origin provide 47 % of the world supply of edible protein (Millward, 1999). Proteins comprise of amino acids arranged in a linear chain joined together by peptide bonds. Quality of proteins is widely evaluated according to the most limiting amino acid. For cereals, the most limiting amino acid is lysine; in proso millet also threonine and tryptophan content is marginal (FAO, 1995). Another method for determining quality of proteins is chemical score suggested by World Health Organization (WHO) in 1985 (Table 9). Chemical score accommodate the human requirements of the essential amino acid for different age groups. Among millets, proso millet has lowest amount or poorest essential amino acid composition (Serna-Saldivar *et al*., 1991) (Tables 10 and 11).

Table 9: Amino acid composition in various millet grains (Lorenz and Kulp, 1991).

Amino acid [g/100 g of protein]	WHO pattern	Millet				
		Proso	Pearl	Finger	Foxtail	Teff
Lysine (Lys)	5.44	**1.5**	2.6	2.0	2.6	3.7
Leucine (Leu)	7.04	**12.5**	17.4	6.4	16.5	8.5
Phenylalanine (Phe)	6.08	**5.5**	4.9	3.2	5.1	5.7
Valine (Val)	4.96	**5.4**	5.7	5.8	5.4	5.5
Tryptophan (Trp)	0.96	**0.8**	2.3	1.5	0.6	-
Methionine (Met)	3.52	**2.2**	2.5	2.3	2.0	4.1
Threonine (Thr)	4.00	**3.0**	4.9	2.7	3.8	4.3
Isoleucine (Ile)	4.00	**4.1**	4.3	3.7	4.2	4.0
Histidine (His)	-	**2.1**	2.1	0.3	3.1	3.2
Chemical Score	100	**27.6**	47.8	36.7	47.8	68.0

Table 10: Amino acid composition in proso millet grain, various authors.

	Phe	His	Ile	Leu	Lys	Met	Thr	Trp	Val
	[g/100 g of protein]								
Lorenz and Kulp, 1991	5.5	2.1	4.1	12.5	1.5	2.2	3.0	0.8	5.4
Serna-Saldivar and Rooney, 1995	5.2	2.2	4.5	12.9	2.2	2.0	2.7	0.9	5.1
	Asp	**Glu**	**Ala**	**Arg**	**Cys**	**Gly**	**Pro**	**Ser**	**Tyr**
	[g/100 g of protein]								
Serna-Saldivar and Rooney, 1995	5.5	20.5	9.3	4.4	1.7	2.2	7.2	6.3	3.9

Asp = Aspartic acid, Glu = Glutamic acid, Ala = Alanine, Arg = Arginine, Cys = Cysteine
Gly = Glycine, Pro = Proline, Ser = Serine, Tyr = Tyrosine

Protein digestibility is an important attribute for a good quality of proteins. Biological value (BV) determines proteins according to nitrogen balance; it is directly related to the efficiency of protein utilisation. These methods include determination of protein efficiency ratio (PER), net protein utilization (NPU), and true protein digestibility (TDP) (FAO, 1995). Some of these characteristics of proso millet grain and other millets are presented in Table 11.

Table 11: Protein characteristics of millets grain (FAO, 1995).

	TPD	BV	NPU
		[%]	
Proso millet	99.3	52.4	52.0
Pearl millet	95.3	62.2	59,3
Foxtail millet	95.0	48.4	46.3
Barnyard millet	95.3	54.8	52.2

The storage proteins create up to 80 % of the total proteins. They are strictly genotypic, thus they are used for varietal identification (Lookhart, 1991). Prolamins and glutelins comprise the bulk of the storage proteins (Table 12); they are located within the

developing grain in protein bodies. The protein bodies in the starchy endosperm can be disorganized during the later stages of grain maturation, it results in a continuous proteinaceous matrix surrounding the starch granules. Proteins associated with the starch granules have a huge impact to the milling properties of the grain and to the functional properties (Shewry, 1996).

Table 12: Protein fractions in proso millet grain (Serna-Saldivar and Rooney, 1995).

Protein Fraction [%]			
Albumins	**Globulins**	**Prolamins**	**Glutelins**
9.0	10.9	31.0	8.0
8.3-9.7	10.5-11.3	25.1-36.9	7.7-8.3

Besides storage proteins, grains contain proteins with key structural or metabolic roles. These proteins can be present in cell walls and membranes or they may contribute to resistance mechanism. Protecting cereal proteins include hydrolytic enzyme inhibiting digestive enzymes of pests and pathogens; hydrolytic enzymes are irreplaceable for utilizing the grain storage reserves (Shewry, 1996). Amylases, proteases, and hemicellulases accumulate in grain during its development (Serna-Saldivar and Rooney, 1995).

Inorganic substance

The inorganic components of the grain include water and minerals. These components contribute to the nutritional and other value of the grain. Grains contain free (easily removed by drying) and bound water, both contributing to factors such as cereal quality, taste and storage properties (Fujino *et al*., 1996).

Proso millet is important source of minerals (Serna-Saldivar and Rooney, 1995) (Table 13), although they represent about 1.5 % of the grain. Minerals are distributed unevenly in the grains. They are abundant in the outer layers, so they decrease to much lower levels on milling. Phosphorus is the most abundant mineral being followed by potassium whereas calcium, sodium and iron are present in low amounts. Most cereal minerals are in an organic form and some in inorganic forms, mainly alkaline metals. Phosphorus, the major mineral in cereals, actually exists in organic forms as phytin, phospholipid, nucleotide or some other compound. The phosphorus in phytin represents 60-80 % of the total phosphoruscontent in cereal grains (Fujino *et al*., 1996).

Table 13: Mineral composition of proso millet, various authors.

	K	Mg	Ca	Na	Fe	Mn	Zn	Cu
	[%]			[ppm]				
FAO, 1995	0.21	0.12	0.01	46.90	33.10	18.10	17.20	8.30
Serna-Saldivar and Rooney, 1995	0.32	0.14	0.02	-	52.00	18.10	17.20	8.30
Oelke *et al*., 1990	-	-	0.05	-				
Léder, 2004	0.32		0.02		52.00	14.00	17.20	8.30

Cereal grains are important source of B vitamins (Table 14), especially thiamin, riboflavin, niacin, and pyridoxine. Vitamins are concentrated in aleurone layer and germ (Serna-Saldivar and Rooney, 1995). Generally the water-soluble vitamins are found more in the outer parts of the grain (Fujino *et al*., 1996) and the decortication of these outer tissues reduced their amount (Serna-Saldivar and Rooney, 1995). Vitamin C (ascorbic acid) can be present in noticeable amount during germination, but in the mature grains is usually not detectable. Cereals are low in lipid content, thus they tend to be low in the fat-soluble vitamins (Fujino *et al*., 1996). The main fat-soluble vitamins in cereals are tocopherol (E group) and carotenoids (provitamin A group, retinol), especially the former. Liposoluble vitamins are concentrated in the germ (Serna-Saldivar and Rooney, 1995).

Table 14 Average composition of B-complex vitamins in proso millet

	Thiamine	Niacine	Riboflavin	Panthotenic acid	Choline
	[mg/100g]				
Oelke *et al*., 1990[a]	0.66	5.33	0.15	0.75	79.56
Serna-Saldivar and Rooney, 1995[b]	0.63	1.82	0.22	1.10	

Antinutritional

Toxic and anti-nutritional substances are found in cereals. These factors modify the nutritional value of the individual grains, and some of them have very serious consequences (FAO, 1995). Natural toxins (of cereal origin) are absorbed from soil (copper, zinc, cadmium) or from air (lead) to the various tissues; they can be translocated in grains. Environmental contaminants are more likely to be found on the grain surface (Fujino *et al*., 1996). Proso millet contains up to 0.5 % of anti-nutritional components (FAO, 1995).

Widely distributed polyphenolic compounds have a role as defence chemicals, protecting the plant from predatory attacks of herbivores, pathogenic fungi and parasitic weeds or insect attack (Leszczynski *et al*., 1989). Phenolic compounds can decrease digestibility, but can decrease the bioavailability of metal ions (Fujino *et al*., 1996). In the

cereal grain they can occur in the form of phytates, enzyme inhibitors, polyphenols and tannins and saponins. Phytate content in proso millet ranged from 0.17 to 0.47 % (Klopfenstain and Hoseney, 1995), tannin content varies from 0.06 to 0.18 %. Dark-grains varieties contain more tannins (Shahidi and Naczk, 2003).

1.1.8. Other millets

Millets species

Pearl millet, *Pennisetum glaucum*, ranks as the world's fourth most important tropical food cereal (FAO, 1995); it is the most widely grown millet throughout the world (Serna-Saldivar *et al*., 1991). It is known as spiked millet, cattail millet, bajra (in India) and bulrush millet (FAO, 1995). Pearl millet originates in savannah of tropical western Africa (Oelke *et al*., 1990), it is excellently adapted to drought and nutrient-poor, sandy soils (Baker, 2004). *Eleusine coracana* Gaertn., known as finger, African millet, koracan, ragi, wimbi, bulo and telebun (FAO, 1995), is the only species of economic importance of the tribe *Chlorideae* (Baltensperger, 1996). In contrast to most millets, finger millet is generally grown in moist climate, mild weather and a considerable amount of rainfall (Baker, 2004). It is widely cultivated in tropical East Africa and Asia, also on rainy slopes and upland areas of Himalayas up to 2 300 m elevation (Baltensperger, 1996). Foxtail millet, *Setaria italica*, is one of the oldest cultivated crops; it was the most important plant food in the Neolithic culture in China (Baltensperger, 1996). Foxtail millet, also called Italian, German Hungarian or Siberian millet (FAO, 1995), ranks second position in the total production of millets. It originates from China or central Asia and it is widely cultivated in China, India, Russia, Japan and the United States. Foxtail millet is distributed in semi-arid regions in temperate, subtropical, and tropical zones. It prospers well in mountainous, plains regions at elevations over 1500 m (Baltensperger, 1996).

Panicum – other cultivated species

Panicum antidotale Retz., Blue panicgrass, is species of south and south-west Asia origin. It is grown as a pasture and fodder crop. *Panicum coloratum* L. (kleingrass) is a polymorphic species native to tropical Africa. It is widely used as grazed pasture or for erosion control. Two botanical varieties are accepted, var. *coloratum*, which includes all cultivated forms. It has evolved ecotypes adapted to a wide range of soils, from lighter sandy soils to, in the case of var. *makarikariense*, heavy clays. *Panicum maximum* Jacq. (Guinea

Grass) is native to tropical and subtropical Africa, but became naturalized in Central and South America in the early colonial period. It produces high yield of palatable mass suitable for pasture, fodder, good quality hay and manure. *Panicum virgatum* L. (switch grass) is widespread in the USA, also extending into Mexico and Central America. It has high potential for biomass production (Parrish and Fike, 2005).

1.2. *Avena sativa* L.

Oats, members of *Avena* tribe, have been originally an unwanted weed in wheat and barley culture (Moore-Colyer, 1995). Although oats represent only minute proportion of cereal production, grains of oats have been used as livestock and human foods since ancient times (Gibson and Benson, 2002). The whole plants have been used as pasture, hay or silage, and straw for bedding for livestock (Schrickel, 1986). Food processing by-products can be used as industrial raw materials. Oat is annual widely grown in temperate and sub-tropical regions, but is very suitable for high-altitude tropic regions (Gyeltshen, 2004). Oats can be used both in intensive agriculture systems and in low-input systems, such as organic agriculture (Moudrý and Strašil, 1996).

1.2.1. Origin

According to archaeological discoveries, oats are mainly Asiatic in origin (Magness *et al*., 1971), specifically in the Middle East near the Mediterranean Sea (Schrickel, 1986). The oldest evidence came from Egypt among remain of the 12th Dynasty (about 2,000 B.C.), but oats were not actually cultivated here, they were weeds. In the Central Asia, Greeks and Romans considered oats to be a diseased version of wheat, they used oats only for feeding of horses. As a weed contaminant of wheat or barley, oats were introduced into the Europe (Moore-Colyer, 1995), where the first cultivation has appeared during the Bronze Age among Germanic and other tribes (Gibson and Benson, 2002).

1.2.2. Economic importance

Oats rank seventh in world cereal production following wheat, maize, rice, barley, sorghum and millets. Oats represented only 1 % of total world cereal production in 2006 (FaoStat, 2007); 23.1 mil t. Developing countries represented 13.4 % of total world production, i.e. 3.10 mil t in 2006 (FaoStat, 2007). The major producers, Russian Federation (4.88 mil t), Canada (3.60 mil t) and the USA (1.36 mil t.), together represented 47 % of the world production in 2006. The Czech Republic produced 0.16 mil t in 2006 (FaoStat, 2007).

1.2.3. Uses

The primary use (75-78 %) of oats have been as forage crop and a feed grain (Cuddeford, 1995; Schrickel, 1986) rather than a human food (18 %) (Webster, 1986). In many countries oats are grown for horse feed (Al Jassim, 2006). Horses and mules prefer oats

to other grains (Gibson and Benson, 2002). The trend in recent time is to feed oats to young stock and poultry (Gibson and Benson, 2002; Pettersson and Åman, 1993; Rodiek and Stull, 2007; Särkijärvi and Saastamoinen, 2006); in poultry they reduce cannibalism (Choct and Haritini, 2005; Hoffman, 1995). Oat straw is very palatable and nutritious, more than wheat straw (Greenhalgh and Reid, 1971; Magness *et al*., 1971).

Food products from oats include oatmeal, oat flour, natural cereals, meat product extenders, cookies and breads, granola, baby food, oat bran, oat milk (Hoffman, 1995) meat extender in hamburger (Inglett *et al*., 1994; Haydanek and McGorrin, 1987); oatmeal based products are the greatest portion of the hot cereal industry (Webster, 1986).

A food processing by-products, oats hulls, are used in chemical manufacture, industrial products (Gibson and Benson, 2002) or as a fuel power plants (Thomas and Ingledew, 1995). Oats hulls are a basic material for producing furfural (Brownlee and Miner, 1948; Bryner *et al.*, 1936; Dunlop, 1948; LaForge, 1924), and are a component in the production of a number of important industrial products, e.g. nylon, lubricants oil, butadiene, phenolic resin glues, and rubber treat materials (Gibson and Benson, 2002). Cosmetic and pharmaceutical industry (Aburjai and Natsheh, 2003; Dull, 1997; Hart *et al*., 1998; Wood and Beer, 2002) exploit anti-inflammatory and hypoallergenic properties of oats; oat grains or straw appear in shampoos, dusting powders, moisturizers, cleansing bars, breast implants, and in astronaut suits. In food industry, oats have been used as an antioxidant (Gray *et al*., 2002; Peterson *et al*., 2001; Xing and White, 1997) and a stabilizer in dairy products (Brennan and Clary, 2005; Gibson and Benson, 2002; Volikakis *et al*., 2004).

1.2.4. Botanical characteristics

Oats have a fibrous root system concentrated in the top soil layer and maximum depth of 80-195 cm (White, 1995).

Erect, hollow stem with 2-8 nodes (White, 1995) can reach 0.6-1.6 m. Terete mid-culm nodes are glabrous, exposed or hidden by the leaf sheaths (White, 1995).

Leaves are 15-30 cm long and 0.6-1.2 cm wide. Leaf sheaths are not keeled. Leaf blades are flat, glabrous, linear, and without grooves; veins are equally striate and margins are membranous and smooth. Membranous ligules are 2.5-3.0 mm long and they are usually obtuse or truncate (White, 1995).

The inflorescence is a terminal diffuse panicle (Figure 4), 15-30 cm long (Duke, 1983). The spikelets of common oat have one to three (rarely four) florets, typically on top of

the branch. They are of cuneate shape and are 18-32 mm long and 20 mm wide (Suttie, 2001). Spikelets of naked oat are multiflorous (usually 7-12 florets per spikelet) (Valentine, 1995). Florets are bisexual or one or two may be reduced and male or sterile (Suttie, 2001). Glabrous lemma is 7-veined. Palea is shorter then lemma and its both veins are covered with hairs. Lemma and palea tightly enclose the grain of common oat. Lemma of naked oats is thin, under-lignified and papery (Valentine, 1995).

Figure 4: Panicle and spikelet of common oat (Watson and Dallwitz, 1992).

Oat grain characteristics

Oat caryopsis are often called groat and according to variety can be husked or huskless. Huskless grains of naked oats have non-lignified lemma, which is less tightly adhering to grain (White, 1995). Common oat grain is oblong, ventrally compressed (Figure 5) and is approximately 9.5-14.0 mm long and 1.8-3.5 mm wide; huskless oat grain is smaller (Doehlert *et al*., 2006). WTS is about 30 g. The surface of the grain is dense covered with bristles (White, 1995); the trichomes are hollow and single-celled (Fulcher, 1986).

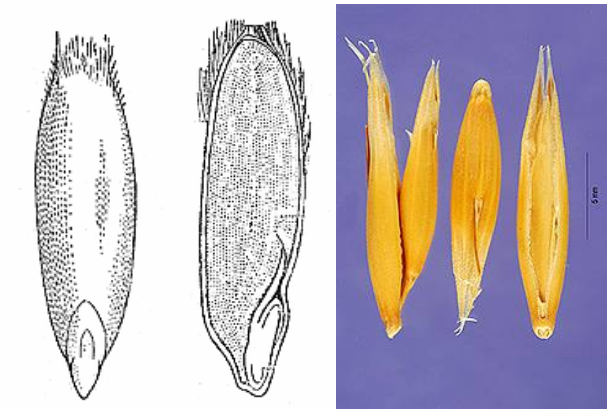


Figure 5: Grains of common oat (Watson and Dallwitz, 1992; Anonym, 2007).

Oats embryo is about 3.4-4.3 mm long and comprises approximately 2.6-3.7 % of the whole grain weight; the endosperm to embryo ratio is generally 151:1 (White, 1995).

The endosperm in oats accounts approximately 50-80 % of the grain weigh (White, 1995). Walls of cell endosperm are composed of two layers: inner large layer consisting of soluble polysaccharides and outer thin layer of insoluble polysaccharides (Miller and Fulcher, 1995). Both layers are rich in mixed linkage β-glucans (Miller *et al*., 1995).

1.2.5. Ecology

Oats are one of the most versatile of the cereals, they require humid, cool climate with constant water supply. Its growing area ranges between 35° and 65°N in the northern hemisphere (Stevens *et al*., 2004) and 23° and 30°S in the southern hemisphere (Forsberg and Reeves, 1995). Oats are frost tolerable in the early stages; optimum annual temperature is 5° to 26°C (Duke, 1983). In tropics, oats are suitable for growing at high altitude up to 2800, as fodder crop they can be grown up to 4000 m (Gyeltshen, 2004).

Oats grow on wide range of soils. They prefer light, well-drained soils, but can tolerate heavy clays and nutritionally poor soils. Oats prefer neutral to slightly acid soils (optimum pH is about 5.3-5.7), but can tolerate acid soils with pH 4.0 (Moudrý, 2005). Oats are less salt tolerant than other common cereals (Bower and Tamimi, 1979).

Oats have a greater water requirement and a better tolerance to water-logging than wheat or barley (Cannel *et al*., 1985; Watson *et al*., 1976).

1.2.6. Agronomy

Oats are long-day crops grown particularly in subtropical and temperate zones. In subtropics, oats are grown in the winter, in temperature regions are seeded as early in the spring as possible, which can help escaping summer drought and heat. Oats germinate at about 1-2°C (Moudrý, 2005), well established seedlings are cold tolerable. Oats prefer firm seedbed at average depth of about 2-5 cm. Seeding rate depends on the grain dimension of an oat variety; it varies from 70-220 kg/ha (Forsberg and Reeves, 1995). In some areas, oats are under-seeded in double cropped soybeans (Burnside and Carlson, 1983; Kaplan and Brinkman, 1984; Moomaw, 1985) or other legume (Carr *et al*., 1998; Caballero *et al*., 1995; Guillard and Allison, 1985) to improve growing conditions, to provide support to the legume and protect against weeds.

Nitrogen is usually applied before or at seeding. Oats require less potassium than other crops, they respond to phosphate and potassium combined fertilizers. Very common lacking micronutrient is manganese (Forsberg and Reeves, 1995). Oats are more sensitive to some herbicides then other cereals (Buchholtz, 1965; Jettner *et al*., 1999).

Oats suffer from a wide range of diseases, pests and disorders. The most important foliar diseases of oats occurring worldwide are powdery mildews, crown rusts, black stem rusts, and leaf blotch. As in other small-grain cereals, oats are damaged by frit flies, especially in spring oats. Other problems, occurring mainly in Europe and Northern America, are cereal cyst and stem nematodes, slugs, letherjackets, and cereal leaf beetle (Clifford, 1995).

1.2.7. Oat grain composition

Oats are one of the most nutritious cereal (Butt *et al*., 2008); they have comparatively high protein quality, the highest level of fat of any of the cereal grains with a favourable ratio of polyunsaturated to saturated fats, and many vitamins and minerals. High soluble fibre (especially β-glucan) can lower the blood-serum cholesterol (Bell *et al*., 1999; Gibson and Benson, 2002; Marlett, 2001; Slavin, 2001). Since oats are generally consumed as a whole grain, these nutrients are not lost in processing (Schrickel, 1986); the preference of whole-grain flour or flake is the exception in comparison to most other cereal grains (Webster, 1986). Average oat composition is presented in Table 15.

Table 15: Composition of oat grain, various authors.

	Protein	**EE**	**NFE**	**Ash**	**Fibre**
	[% in dry matter]				
Matz, 1991	16.9	6.9	66.3	1.7	5.2
Webster, 1986	15.2	7.6	65.3	1.9	8.6
Moudrý, 2005 (naked oats)	16,5	6.7			2.2
Welsch, 1995	11.3	5.1	57,8	3,1	
Asp *et al*., 1992	15.9	7.0	63.2		9.7

Lipids

Oats contain the highest lipid content, on average 6-8 %, that is not found in any other cereal grain (Deane and Commers, 1986); naked oats are higher in lipids than husked oats (Givens *et al*., 2003; Givens and Brunnen, 1987). The lipid distribution within the oat grain is irregular, the highest lipid concentration is in the embryonic axis and the scutellum (Table), but about 50 % up to 90 % (Banaś *et al*., 2007) of the lipids appear in the endosperm. Lipids in oats are of very high quality; lipids are very highly unsaturated; they contain significant

amounts of linoleic acid (Zhou *et al*., 1998a). High lipid content and high lipid-related enzymes can cause rancidity problems.

Fatty acid distribution in oat glycerides is not regular, Youngs *et al*. (1977) reported only 2 % 1,3-diglycerides and 1 % of 1,2-diglycerides. Triglycerides appear mainly in the embryo (Youngs *et al*., 1977). Palmitic, oleic, and linoleic acids together comprise 95 % of the fatty acids (Zhou *et al*., 1998a) (Table 16). The most common saturated fatty acid is palmitic acid (Youngs *et al*., 1977), the most common unsaturated acids are oleic and linoleic acid (Zhou *et al*., 1998a). Myristic (14:0), stearic and linolenic acids occur in lesser amounts (Youngs *et al*., 1977); palmitoleic, arachidic (20:0), gadoleic (20:1), behenic (22:0), erucic (22:1), lignoseric (24:0) and nervonic acid (24:1) in minute amounts have been reported by Zhou *et al*., (1998) in some Australian varieties.

Table 16: Fatty acid composition in oat grain, various authors.

	14:0	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**
				[%]		
Youngs *et al*., 1977	0.6	18.9	1.6	36.4	40.5	1.9
	0.4-0.8	16.2-21.8	1.2-2.0	28.4-40.3	36.6-45.8	1.5-2.5
Zhou *et al*.,1998a		17.0-20.0		38.0-43.0	36.0-40.0	

Carbohydrates

Oats contain about approximately 60 % of starch. Oat starch consists of 16-23 % of amylose and it contains, in comparison to other cereals, higher lipid content (1.2-1.6 %). Oat starch granules are smaller than in other cereals; the average granule diameter is 5-12 μm (Hoover and Senanayake, 1996). Shape of the granules is irregular, often of a polyhedral configuration (Paton, 1986). The surface of granules is smooth with no fissures or pores (Hoover and Senanayake, 1996). The granules are associated in clusters of individual granules; aggregated starch granules exist in the size range of 30-60 μm in diameter. These aggregates are located closely-up to protein bodies. Oat starch functional characteristics are very similar to that of corn starch, but exhibit higher water absorption at room temperature (Table 17). Oat starch gels are more elastic, adhesive, and translucent and show greater stability (Paton, 1986).

Table 17: Properties of oat starch (Paton, 1979).

	Iodine Affinity at 30°C [%]	Amylose [%]	Swelling power at 95°C	Solubility at 95°C
Oats (var. with high protein content)	3.19	16.8	23.6	16.8
Oats (var. with low protein content)	3.54	18.6	19.4	26.0
Oat cv. Hinoat	3.26	17.2	23.6	16.8
German varieties	3.52	18.7	22.0	15.0

The crude fibre content of oats is about 11-12 %; it consists of 16.7 % of lignin and 29.4 % of α-cellulose. The highest concentration of fibre is in the hull (MacArthur-Grant, 1986). Oat grain carbohydrates contain about 14 % pentosans, mainly araban and xylan, the higher amount of pentosans can be found in the hull. Oat pentosans are used for commercial production of furfural and related furan compounds (MacArthur-Grant, 1986). Oat β-D-glucan, oat bran's soluble fibre, is presented in the amount of 3.5-5.7 % (Asp *et al*., 1992). This substance has some beneficial properties (Braaten *et al*., 1994; Davis *et al*., 2004; Estrada *et al*., 1999).

The low molecular weight carbohydrates concentration is lower in comparison to other cereals, i.e. about 1.4 %. The most frequently reported carbohydrates in oats include sucrose, raffinose, glucose, fructose, and maltose (Table 18). Besides them, oats contain stychyose and verbascose in bran (MacArthur-Grant, 1986).

Table 18: Free sugar content in oat flour and bran (MacArthur-Grant, 1986).

	Sucrose	Raffinose	Maltose	Stachyose	Verbascose	Fructose	Glucose
				[%]			
Oat flour	0.49	0.21	0.06	0.08	-	0.03	0.57
Oat bran	2.21	0.36	0.03	0.21	0.04	0.05	0.08

Proteins

Thanks to oat protein favourable amino acid balance (Tables 19 and 20); oat protein quality surpasses other cereals (Peterson and Brinegar, 1986). Limiting amino acid in oats is lysine, but its content can be increased by fertilizers (Welch, 1995). Amino acid balance of the most common oat products has been investigated by Pomeranz *et al*. (1973) (Table 21). Oat high-quality protein could be beneficial in some areas, where dietary protein is only of plant origin or replacing soybean meal in livestock feed (Peterson and Brinegar, 1986). Proteins are distributed about equally in bran and starchy endosperm (both about 10 % of proteins), the bran contains about 20 % of proteins. The highest protein content is in the

embryo, i.e. over 30 % (Lasztity, 1996). In the aleurone layer, proteins are concentrated in association with phytin in aleurone protein bodies. These protein bodies develop in vacuoles (Peterson and Brinegar, 1986).

Table 19: Essential amino acid composition in oat grain, various authors.

	Phe	His	Ile	Leu	Lys	Met	Thr	Val
	[g/100 g of protein]							
Robbins *et al*., 1971	5.3 4.9-5.7	2.2 1.2-3.1	3.9 3.4-4.1	7.4 4.8-7.8	4.2 3.2-5.2	2.5 1.0-3.3	3.3 3.0-3.5	5.3 4.9-5.7
Zarkadas *et al*., 1982	5.1	1.8	3.7	7.0	4.4	1.5	3.4	4.7
Pomeranz *et al.*, 1973	5.3-5.4	2.4-2.7	4.2-4.5	7.5-7.6	4.2-5.2	1.5-2.3	3.3-4.1	5.8-6.2
Fulcher, 1986	4.5	2.2	4.5	8.2	3.8	1.9	3.4	6.7

Table 20: Non-essential amino acid composition in oat grain, various authors.

	Asp	Glu	Ala	Arg	Cys	Gly	Pro	Ser	Tyr
	[g/100 g of protein]								
Robbins *et al*., 1971	8.9 8.3-9.9	23.9 21.9-26.9	5.0 4.2-5.5	6.9 6.2-7.8	1.6 0.6-2.6	4.9 4.4-5.5	4.7 3.8-5.8	4.2 3.8-4.8	3.1 2.3-4.4
Zarkadas *et al*., 1982	8.9	21.8	5.3	7.0	3.4	5.5	6.9	5.4	4.1
Pomeranz, 1973	9.2-11.1	20.0-21.6	5.1-5.5	6.3-6.4	0.4-1.7	5.1-6.0	3.1-5.7	4.0-4.5	2.4-2.6
Fulcher, 1986	8.7	20.0	6.9	5.7	0.6	9.2	6.3	4.8	2.6

Table 21: Amino acid composition of commercial oat products (Pomeranz *et al.*, 1973).

Amino acid [g/100 g of protein]	Heavy Oats	Light oats	Groats	Hulls	Flakes
Lys	4.2	5.2	3.9	4.9	4.1
His	2.4	2.7	2.3	2.4	2.3
Arg	6.4	6.3	6.2	6.8	6.0
Asp	9.2	11.1	9.0	10.5	9.0
Thr	3.3	4.1	3.1	4.1	3.1
Ser	4.0	4.5	3.9	4.6	4.0
Glu	21.6	20.0	22.4	20.3	22.7
Pro	5.7	3.1	6.2	2.4	6.1
Cys	1.7	0.4	2.0	0.5	1.7
Gly	5.1	6.0	5.0	6.1	5.0
Ala	5.1	5.5	5.0	5.4	5.0
Val	5.8	6.2	5.7	6.4	5.8
Meth	2.3	1.5	2.5	1.5	2.4
Ile	4.2	4.5	4.3	4.5	4.3
Leu	7.5	7.6	7.4	7.9	7.5
Tyr	2.6	2.4	2.5	2.9	2.1
Phe	5.4	5.3	5.5	5.3	5.6

Although most cereals have higher prolamins content, oats along with rice are exceptions - their major storage proteins are globulins, called avenalins (Lasztity, 1996). Oat high molecular weight protein fraction distribution shows Table 22.

Table 22: Distribution of protein fractions in oat grain, various authors.

Authors	Protein Fraction [%]			
	Albumins	**Globulins**	**Prolamins**	**Glutelins**
Peterson, 1976	14.0 10.0-19.0	53.4 52.0-56.0	9.2 7.0-13.0	23.2 21.0-27.0
Peterson and Brinegar, 1986	9.0-20.0	47.0-63.0	4.0-14.0	
Welsch, 1995	14.4-20.1	47.1-53.2	7.2-9.9	21.4-26.7

Trypsin inhibitor founded in oat flour, is thermolabile (Mikola and Mikkonen, 1999) and is completely destroyed by pepsin (McNiven *et al*., 2002). Hydrolytic enzymes are very active during germination; they provide substrates to the developing embryo (Peterson and Brinegar, 1986). Lipases are mainly located in outer pericarp layers of the groat (Ekstrand *et al*., 1992; Martin and Peers, 1953). The oat lipase demonstrates substrate selectivity, the most rapidly reactions were upon triacylglycerols containing oleic, linoleic and linolenic acids (Piazza *et al*., 1992). Lipase extraction and purification have been studied by Martin and Peers (1953) and Peers (1953). Activity of oat lipases has been demonstrated by Sahasrabudhe (1982).

Inorganic substances

Ash content in oat grain is about 3 % (Welch, 1995); oats are a good source of manganese, magnesium, and iron, as well as calcium, zinc and copper (Lockhart and Hurt, 1986). Tables 23 and 24 represent mineral content and distribution in oat grain or its products.

Table 23: Mineral content of oat grain, various authors.

	Ca	**P**	**Na**	**K**	**Mg**	**Cu**	**Co**	**Mn**	**Zn**
			[%]				**[ppm]**		
Lockhart and Hurt, 1986	0.11	0.38	0.02	0.47	0.13	4.7	0.05	45.0	37.0
Welch, 1995	0.52	0.42	0.09	0.36	0.14	4.4		42.0	35.5
Matz, 1991	0.54	0.523	0.04		0.18	4.9			39.7

Table 24: Relative distribution of minerals within the oat groat (Lockhart and Hurt, 1986).

	P	Ca	K	Mg	Mn	Fe	Zn
	[%]				[ppm]		
Bran	1.02	0.11	1.00	0.38	88	90	58
Endosperm	0.26	0.10	0.16	0.07	31	18	24

Oat grains are a great source of water-soluble B vitamins, especially of thiamine, niacin and pantothenic acid (Welch, 1995). Of fat-soluble vitamins, oats contain significant amount of vitamin E (Herting and Drury, 1969). The major portion of the vitamin content is located in the outer bran fraction (Lockhart and Hurt, 1986). Vitamin content of oat grain and its products are demonstrated in Tables 25 and 26.

Table 25: Vitamin content in oat grain (Lockhart and Hurt, 1986).

	Thiamine	Riboflavin	Niacin	Pantothenic acid	Pyroxidine	Folic acid	α-Tocopherol
	[mg/100g]						
Oat groat	0.77	0.14	0.97	1.36	0.12	0.06	
Rolled oats	0.67	0.14	0.98	1.48	0.13		1.94

Table 26: Vitamin and mineral content of rolled oats, various authors.

	Thiamin	Riboflavin	Niacin	Vit. B6	Folic Acid	Biotin	Calcium
	[mg/100g]				[µg/100g]		
Lockhart and Hurt, 1986	0.670	0.110	0.80	0.210	104	13	50
Welch, 1995	0.605	0.120					
Matz, 1991	0.763	0.139	0.961	0.119			

Antinutritional factors

Oats usually contain insignificant amounts of antinutritional components. Oat proteins, in comparison to wheat or barley, are not toxic to individuals with celiac disease (Picarelli *et al*., 2001). Many of phenolic substances are concentrated in the outer layers of oat pericarp (Peterson, 2001; Zieliński and Kozłowska, 2000). These substances, e.g. caffeic acid, ferulic acid (Bratt *et al*., 2003), *p*-coumaric acid, vanillic acid, *p*-hydroxybenzoic, *p*-hydroxybenzaldehyde, vanillin, catechol (Xing and White, 1997), coniferyl alcohol, and avenanthramides (Bratt *et al*., 2003; Dimberg *et al.*, 1993; Peterson *et al.*, 2002), were reported to have antioxidant properties.

1.2.8. Other *Avena* species

The oat species include *Avena abyssinica* Hochst., *A. byzantina* K. Koch, *A. nuda* L., *A. sativa* L., *A. strigosa* Schreb., *A. fatua* L. and others. The most cultivated is *A. sativa*, representing 75 % of the total world production of oats (Schrickel, 1986).

Abyssinian oat, *Avena abyssinica*, native to Ethiopia, can be grown in arid regions and can stand high elevations. It is grown for grains in monoculture or in a mixture with barley (Vietmeyer and Ruskin, 1996).

Red oat, *Avena byzantina*, is adapted to warmer climates than common oats and is more resistant to drought. It is native to Mediterranean area. It is sometimes cultivated for grains; more often is cultivated for hay or pasture (Suttie and Reynolds, 2004).

Black or small oat, *Avena strigosa*, is tropical and subtropical annual cereal, grown as livestock forage, cover crop and green manure. It is widely grown in South America (Stevens *et al*., 2004). It is very rust resistant (Steinberg *et al*., 2005).

Common wild oat, *Avena fatua*, is one of the most aggressive weeds, native to Eurasia. Contemporary wild oat is highly resistant to many herbicides and become an increasing problem (Friesen *et al*., 2000).

Mistaken terms “naked oats” and “huskless oats” include both diploid and hexaploid oats (naked character doesn’t appear at tetraploid level). There is not unity about taxonomy position of naked oats. Diploid naked oats are named as *A. nuda*, *A. strigosa* subsp. *nuda*. *A. nuda* is sometimes synonym of *A. strigosa*, because diploid naked oats is fully interfertile with husked *A. strigosa*. On the other hand, hexaploid naked oat is often referred as *A. sativa* subsp. *nuda*, because huskless and husked hexaploid oats differ in only a single major gene (*N-1*) responsible for the huskless character. Some scientists assume *A. sativa* subs. *nuda* to be incorrect and employ *A. sativa* subs. *nudisativa* (Valentine, 1995). Because all hexaploid huskless oats are usually classified as naked oats, this thesis employs term “naked oat” for huskless hexaploid oats.

For hexaploid naked oats is characteristic, that the husk is threshed during harvesting. Removal of this fibrous husk has a great effect on nutrient and energy content. Huskless oats are very suitable for non-ruminants (pigs, poultry, and human) consumption without processing (Valentine, 1995). Because of a great similarity of naked oat and husked common oat, they are characterized together, separately are mentioned when they differ.

1.3. Grain quality

Quality is judged according to many characteristics. It is a system of distinguishing characteristics or properties of products, which may be assigned different significance depending on the desired and use or type of product. Quality of food is based on a combination of many subjective and objective factors. Quality of cereals is generally divided into physical or technological quality, and nutritional quality. The quality of final products is depended on processing conditions, product characteristics, product performance, and consumer requirements (Peri, 2005).

Physical and technological quality is influenced by morpho-physiological traits. The main important physical properties are grain characteristics. The most important technological properties are processing, milling and cooking quality. Nutritional quality is mainly influenced by chemical composition and the presence of anti-nutritional factors; it is connected with safety of food (Serna-Saldivar and Rooney, 1995).

1.3.1. Effects of agronomy and environmental conditions

In addition to the genetic differences in cereal grain, there are several agronomic factors and environmental influences which have remarkable effects on grain quality (Kettlewell, 1996). Climatic conditions can influence appearance of pathogenic fungi (Doohan *et al*., 2003).

Influence of agronomy and environmental conditions in oats were studied by Moudrý (2005), Frey (1959a, 1959b), Givens *et al*., (2003), Humphreys *et al*., (1994), Ohm (1976), Pendleton and Dungan (1958), and Zhou *et al*. (1998b). These effects on proso millet were investigated by Agdag *et al*. (2001), Anderson *et al*. (1987), Channappagoudar *et al*. (2007), and Nelson (1981).

Growing conditions

Heat stress reduces yield and change grain quality in cereals and its impact depends on the intensity and duration. Photosynthesis and respiration are highly temperature sensitive, which can lead to reduce biomass production and grain filling. The photosynthetic response to low temperature is species and variety dependent. Although optimum temperature for C4 crops is higher, photosynthetic activity for both C3 and C4 plants declines over 40°C (Stone, 2000). Proteins are not as responsive to heat stress as carbohydrates (Kettlewell, 1996). Lipid

fractions are greatly influenced by growing conditions; the temperatures below 12°C induce higher free and bound lipids amounts and greater unsaturation (Beringer, 1971). Cold and frost reduces grain size and baking qualities (Allen *et al*., 2001).

The precipitation influences many aspects of grain quality. Influence of water stress depends on time and duration of it (Morgan and Riggs, 2006). Short or low water stress has negligible impact, because of yield component compensation. Carbohydrates might decrease (Fernandez-Figares *et al*., 2000) due to stomatal closure (Kettlewell, 1996). Protein concentration in grain can slightly increased under water stress, but protein composition is usually stable (Fernandez-Figares *et al*., 2000; González *et al*., 1999; Kettlewell, 1996). Pre-anthesis drought can stimulate late maturing tillers in small-grain millets, which produce immature kernels at harvest time. Waterlogging reduces root growth and leads to nutrient leaching (Kettlewell, 1996).

Agronomy influence

Seeding date influences kernel size and can indirectly influences some characteristics connected with proteins; delayed seeding reduces grain yield (McKenzie *et al*., 2005). Seeding rate has usually little effect on kernel size, test weight or proteins (Kettlewell, 1996).

Grain characteristics and quality are greatly influenced by accessible nutrients; they can be modified by fertilizing. Grain quality is more responsive to nitrogen of all nutrients. Nitrogen is a major component of protein, and nitrogen supply to cereal crops is the principal factor influencing grain protein concentration and protein composition, which can greatly affect the baking quality (Kettlewell, 1996). Nitrogen supply increases protein content in grains, but decrease thousand kernel weights. The effects of nitrogen fertilizing were demonstrated on wheat (Pechanek *et al*., 1997; Johansson *et al*., 2001; Terman, 1979; Woolfolk *et al*., 2002), barley (Birch and Long, 1990, Metivier and Dale, 1977; Baethgen *et al*., 1995), oats (Portch *et al*., 1968; Zhou *et al*., 1998b), sorghum (Iptas and Brohy, 2003) millets (Bailey *et al*., 1980; Stabursvik and Heide, 1974) and proso millet (Turgut *et al*., 2006). Sulphur supply has relatively little effect on protein concentration, but considerably influences protein quality (Flæte *et al*., 2005; Lerner *et al*., 2007; Wooding *et al*., 2000; Zhao *et al*., 1999). Potassium can influence test weight, but can slightly decreases protein content (Zubriski *et al*., 1970). Other nutrients, such as zinc, ferrum, cupper and others can increase in grain by adequate fertilization (Rengel *et al*., 1999).

Too early harvest leads to reduced yields, test weight and colour quality; late harvest causes losses due to shattering, lodging and increasing losses caused by rodents and birds (Oelke *et al*., 1990).

Diseases can have serious consequences for grain quality. Seeding diseases reduce yield and kernel size, root and stem diseases influence nutrient and water uptake and distribution, foliage diseases reduce photosynthesis and indirectly influence grain quality. Pests have very similar impact depending on the location of the injury. Fungal diseases have the major impact on quality (Kettlewell, 1996), but some fungicides have adverse effect on grain quality (Dimmock and Gooding, 2002). Weeds contribute to increased impurity in grains (Kettlewell, 1996). Due to higher competition, weeds decrease grain yield, but have only indirect influence on grain quality (Bell and Nalewaja, 1968). The problem of weed competition was reviewed by Zimdahl (2004).

1.3.2. Effects of processing

Cereal grains are rarely consumed in their raw state; cereal processing is one of the oldest food technologies. All types of processing can influence the nutrient composition of grains (Tables 27 and 28). Decortication influences the amount of important nutrients and nutrient digestabilities. Decortication is often considered to have negative impact by losses of nutrient, and positive impact by reducing levels of fibre and some anti-nutritional compounds. Processed grains are often nutritionally superior to unprocessed grains (Slavin *et al*., 2000), processing can improve colour, yield and texture of final product (Aboubacar *et al*., 2006; Yetneberk *et al*., 2005). Decortication generally leads to losses of lipid, protein, fat, fibre and micronutrients (Lorenz and Kulp, 1991; Köksel *et al*., 1999), but their losses can be compensated by increasing of their bioavailability (Lestienne *et al*., 2007). Decreasing of anti-nutritional factors leads to reducing of total content of phenolic compounds, tannin content and antioxidant activity (Dlamini, 2007). Millets are usually decorticated to remove 12-30 % of the grain. In proso millet decortication leads to losses of carbohydrates fat (from 4.6 to 2.6 %), fibre (5.1 to 0.65 %), ash (2.5 to 0.9 %), calcium (0.04 to 0.01 %), iron (28 to 13 mg), lysine (182 to 143 mg/g N), methionine (129 to 110 mg/g N) (Lorenz and Kulp, 1991), and tannin (Geervani and Eggum, 1989). The protein digestibility can increase (Lorenz and Kulp, 1991). Composition of processed proso millet is presented in Table 25.

Table 27: Effects of some processing (Lintas and Mariani-Constantini, 1991).

		Protein	Fat	Carbohydrate	Ash
Barley	Whole grain	10.6	2.1	57.5	3.1
	Pearled	10.4	1.4	62.2	1.2
Corn	Grain	9.2	3.8	65.5	1.3
	Flour	9.0	2.8	73.1	1.2
Oats	Husked grains	12.6	7.1	61.2	2.9
	Groats	13.9	5.8	-	2.0
Rice	Brown	7.4	2.2	74.6	1.2
	Milled	7.2	0.7	78.4	0.5
Rye	Grain	8.7	1.7	53.5	1.9
	Flour	6.9	0.7	65.7	0.7
Wheat	Hard spring	11.5	2.0	59.4	1.8
	Durum	14.0	2.9	57.9	1.5
	Flour	12.7	1.3	67.6	0.7

Table 28: Composition of common millet and products (Winton and Winton, 1932).

[%]	Starch	Protein	Fat	NFE	Fibre	Ash
Rough millet	62.56	11.56	3.29	62.97	10.00	2.88
Bran	19.03-27.83	6.68-6.25	2.33-2.38	19.50-28.58	52.50-43.78	8.72-9.36
Polish	34.12-41.59	18.06-18.37	16.50-18.48	35.02-42.61	6.38-11.07	7.14-8.44
Decorticated millet	72.56-74.40	11.40-13.06	2.81-2.84	72.99-75.14	0.23-0.46	0.88-1.26

Grains are usually ground to flour; milling removed all of the pericarp, seed coat and nucellus, and practically whole aleurone layer and embryo. Flour yield of red proso millets is 79.2-82.4 % and of white proso millet 82.1-87.1 % (Lorenz and Hwang, 1986**)**. The concentration of essential nutrients decreases by milling (Pederson and Eggum, 1983a, 1983b, 1983d, 1983e). Protein content is reduced, but true protein digestibility increases. Minerals can be lowered to 50 % or more, the most affected minerals are zinc and phosphorous (Pederson and Eggum, 1983c). Vitamins can decrease to 70-80 % (Hegedüs *et al*., 1985). Milling leads to decrease of polyphenols and phytic acid (Chowdhury and Punia, 2006).

Oats are generally eaten as flakes, made from whole groats passed through a grain mill with a flaker attachment. To increase shelf life of processed oat products, heat treatment is applied to inactivate hydrolytic enzymes (Lehtinen *et al*., 2003). Temperature plays an important role in the processing of cereal products. Heating or boiling can lead to reduction in heat labile nutrients, e.g. changes in conformations of proteins, their polymeric state, and their interaction behaviour and gelatinisation of starch are affected by temperature. Heating results in nutrients depletion, especially proteins, phosphorus and magnesium; mineral content is not

so affected (Ebuehi and Oyewole, 2007). Lysine content is significantly reduced during heating (Sharma *et al*., 2004). Natural antioxidants have low heat resistance, and temperature over 80°C can destroy their antioxidant properties (Zadernowski, 1999). Heating or boiling can reduce levels of mycotoxin contamination in cereal products (Adegoke *et al*., 1994).

Fermentation of food is widely used in traditional food technologies throughout the world to change texture of food, create enhanced flavour, improve nutritional quality, and digestibility (Keregero and Kurwijila, 1987). Fermentation decreases total starch, but improves its digestibility and improves digestibility of fat (Skrede *et al*., 2002). Protein quality is not affected by fermentation (Ene-Obong and Obizoba, 1996); the amino acid composition can be slightly improved (Asiedu *et al*., 1993). Fermentation leads to a significant improvement in mineral absorption (Agte *et al*., 1999; Skrede *et al*., 2002) and a significant reduction (up to 50 %) in total polyphenols and phytic acid (Reddy and Pierson, 1994; Sharma and Kapoor, 1996).

Sprouting is very important traditional processing method; it induces significant decreases in antinutritional factors and storage starch decomposition. Sprouting increases protein digestibility (Mbithi-Mwikya *et al*., 2000), and vitamin content (Asiedu *et al*., 1993). It leads to losses of dry matter, gross energy and triglyceride content (Chung *et al*., 1989). Sprouting was reviewed by Wigmore (1986). Nutritional changes of oats during germination were studied by Gabrovská *et al*. (2004).

1.3.3. Effects of storing conditions

Cereal grains are subject to quality loss during storage mainly due to deterioration; post-harvest losses can comprise 5-20 % in cereals (Chakraverty *et al.*, 2003). The effects of deterioration can be considerably diminished through careful stored grain management. The influences of stored cereal grains can be divided to abiotic influences (moisture, temperature, storage period, atmospheric composition) and biotic influences (grains themselves, debris, moulds, pests) (Mills, 1996). Although the major part of the post-harvest grain losses is caused from pests infestation (Tipples, 1995), temperature and moisture largely determine the duration of safe storage (Rajendran, 2003).

During deterioration, some changes in the grain composition occur. Losses of vitamins, the total sugars, protein and starch digestibility (Rehman, 2006), increasing the level of phosphorous components (Rajendran, 2003), and last but not least, development of rancidity. Liberating the free fatty acids due to rancidity is highly sensitive indicator of grain

deterioration (Rajendran, 2003); these problems will be described in detail in the separate chapter. Changes of nutritional quality lead to changes of processing qualities (Daniels *et al*., 1998; Perdon *et al*., 1997; Posner and Deyoe, 1986; Zhou *et al*., 2003).

Water content, both of grains and atmosphere, and temperature are by far the most important factor influencing stored grain quality (Tipples, 1995); their influence in the case of rancidity is given later. Unsuitable temperature and water content in stored grains increases grain respiration (Dealey, 1975; Dillahunty *et al*., 2000), protein and starch digestibility (Rehman, 2006; Rehman and Shah, 1999; Rehman *et al*., 2002) and vitamins content (Sobolev *et al*., 1987; Sudesh and Kapoor, 1994).

Storage period depends on other factors, moisture, temperature, storage structures, and others. The duration of safe storage is outcome of the maximum moisture level and optimal temperature (Mills, 1996). The composition of the intergranular atmosphere influence grain quality due to reducing moulds, insects and mites (Mills, 1996), and determining the type of their metabolism (Rajendran, 2003). The most widely used techniques are modified atmosphere storage, hermetic storage, and controlled atmosphere storage. They are used to lengthen shelf life of agricultural and food products, especially fresh fruits (Kader *et al*., 1989) or grains (Bell, 1993; Richard-Molard, 1990). By reducing the oxygen content, the rate of oxidation can be significantly reduced (Robertson, 2006).

Debris, a waste material originating mainly from the field, includes cereal stalks, chaff, shrunken or underdeveloped seeds, weed seeds, broken kernels, fine particulates, and dust. Debris can cause spoilage or heating in stored grains (Mills, 1996). Post-harvest storage moulds represent the major causes of stored grain deterioration (Tipples, 1995). Moulds cause adverse grain aggregation, heat-damage, production of toxins and allergens, and quality changes, such as musty odours reduction in germinability and discolouration. Occurrence and type of mould strain depends on relative humidity and temperature required for their growth and development (Mills, 1996). Stored grains and their products worldwide are affected by several hundred different species of insects, of which about 50 species are serious pests. Pests influence the storage grains by feeding grains, contaminating by faeces and body parts and they contribute to the entrance of moulds and mites into grains (Mills, 1996). They usually contamine a great deal more than they eat (Tipples, 1995). Rodents and birds influence stored grains in the same way as insect (Mills, 1996).

1.4. Rancidity

Like the other organic materials, cereal grains are subject to deterioration; the principal form of the deterioration, in the case of oils and fats, is rancidity (Deuel, 1957). The word "rancid" is derived from the Latin word for "stinking", thus rancidity is associated with adverse quality factors and a resultant loss of acceptability. Hamilton (2005) defined rancidity as the subjective organoleptic appraisal of the off-flavour quality of food. The term "off-flavours" comprises wide variety of changes of flavours, odours, colours, taste and functional properties (Morris *et al*., 2004); human taste-buds are highly sensitive to off-flavour compounds (Sanders, 1983). The main sources of off-favours in foods are environmental contaminants (Morris *et al*., 2004), the growth of spoilage microorganisms (Jensen and Grettie, 1933; Montel *et al*., 1998), oxidation or enzymatic decomposition of lipids. Foods mainly associated with this type of deterioration include meat with high fat concentrations, such as fish (Ackman, 1967; Harris and Tall, 1983), dairy products (Allen, 1983; Duncan *et al.*, 1991), processed food (; Landers and Rathmann, 1981; Lindley, 1998), and of course, cereal grains. Seeds have a number of protective features to survive adverse conditions; healthy and sound grains may be expected to retain its usefulness as a food source over many months or even years. According to Galliard (1983), in cereal grains, development of rancidity occurs mainly due to disease or damage.

In general, reactions involved in producing rancidity may be described as either hydrolytic or oxidative character. Hydrolytic rancidity is connected with enzymic activity, while oxidative rancidity originates from the interaction of oxygen (Deuel, 1957). Both types occur in cereal grains and their products (Galliard, 1983). Hydrolytic rancidity is caused by hydrolysis of the triglycerides and the liberation of free fatty acids in the presence of moisture. Oxidative rancidity is caused by oxygen attack on the lipid (Rossel, 1983).

1.4.1. Hydrolytic rancidity

Hydrolytic rancidity is characterized by liberation of free fatty acids form the parent fats (Rossel, 1983) results from an enzymatic hydrolysis of fat (Morris *et al*., 2004). Hydrolysis of carboxylic ester bonds of triglyceride, most vulnerable spots in the molecule (Deuel, 1957), releases up three molecules of free fatty acids (Galliard, 1983) (Figure 6). These liberated acids can cause a distinctive undesirable odour and taste (Morris *et al*., 2004).

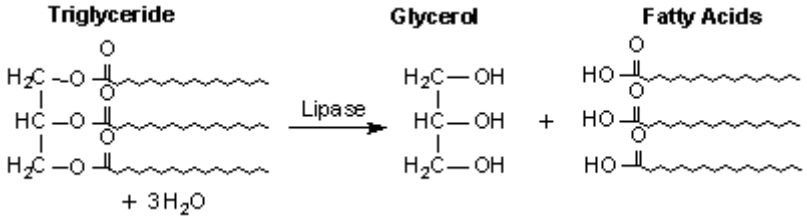


Figure 6: Diagram of hydrolytic rancidity (O´Mahony and Peters, 1987).

The hydrolysis is catalyzed by acid, alkali or by lipolytic enzymes; for industrial purposes the reaction is carried out under high temperature and high pressure (DeMan, 1992). According to Galliard (1983), hydrolytic rancidity in cereal grains and cereal products is more likely to be due to enzyme activity than to acid- or base-catalysed hydrolysis. Among enzymes, lipase activity appears to be an important factor determining the intensity of the hydrolytic process (Al-Kahtani, 1985; Goffman and Bergman, 2003). These enzymes are concentrated in peripheral tissue and germ, but activity of lipases can also originate in bacterial contamination (Hamilton, 2005) or from fungal (moulds) contamination (Galliard, 1983). Thus, hydrolytic rancidity is generally caused by a combination of microorganisms and moisture (Rossel, 1983).

The off-flavours are characterized by liberated fatty acids, especially their molecular weight. The shorter the chain length the more soluble the fatty acid in water, leading to a lower threshold for tasting it in the mouth; most noticeable off-flavours are caused by fatty acids with a chain length below C_{12} (Rossel, 1983), C_{16} and longer are non volatile (Becker, 1992). Thus, according to Hamilton (2005), separate hydrolytic rancidity is much more important in animal fats than for vegetable fats. The enzymatic hydrolysis is followed by the oxidation of liberated free fatty acids, which produce volatile compounds with typical rancidity off-flavours (Becker, 2002).

1.4.2. Oxidative rancidity

Oxidative rancidity is clearly caused by atmospheric oxygen attack on the lipids (Rossel, 1983) (Figure 7), catalyzed by a range of agents (Galliard, 1983). The first product of the reaction of fat and oxygen is an intermediate, a lipid hydroperoxide and peroxides (sometimes referred to as the "hydroperoxide hypothesis") (Loury, 1972). The hydroperoxides are relatively involatile and do not produce any off-flavours (Hamilton, 1983); but are very unstable and break down to volatile aldehydes, hydrocarbons, ketones and alcohols (Loury, 1972) (Figure 7), which may degrade taste and odour (Rossel, 1983). The

majority of compounds that are subject to autoxidation are unsaturated compounds (Chan, 1987) and isoprenoid compounds (Galliard, 1983). Cereal lipids are potentially susceptible to oxidation, because of the majority (30-60 %) of the unsaturated and polyunsaturated fatty acids (Galliard, 1983).

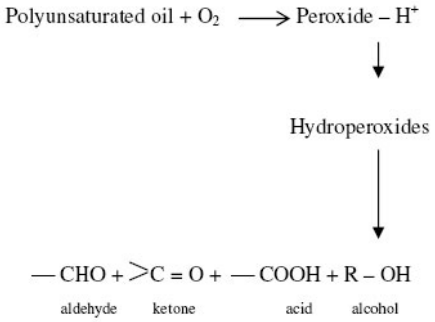


Figure 7: Diagram of oxidative rancidity (Onyeike and Oguike, 2003).

According to Hamilton (1983), the production of the hydroperoxides can occur by one of three mechanisms: the classical free radical mechanism, photo-oxidation and enzymic lipoxygenase oxidation. Rate of oxidation is dependent on several factors, including temperature, nature of the substrates and presence of inhibitors or catalysts (Min and Boff, 2002). The exact products formed depend on the fatty acids present and the initially formed hydroperoxide isomers.

Oxidation can alter the flavour and nutritional quality of foods and produce toxic compounds (Min and Boff, 2002); even at low levels of oxidation, the off-flavour can make the foods less acceptable or unacceptable to consumers. The ability of perceiving the off-flavours is subjective and varies from person to person (Hamilton, 2005).

Radical route

The major reaction in the formation of the hydroperoxides is a free-radical chain reaction (Hamilton, 2005). This sequence of reactions, referred to as autoxidation, is autocatalytic (Chan, 1987), is characterized by the production of free radicals $R^{\bullet}$ from lipid molecules and their interaction with oxygen in the presence of a catalyst. The processes are generally considered to occur in three phases: an initiation or induction phase, a propagation phase, and a termination phase. The mechanism of lipid autoxidation has been postulated by Farmer *et al*., (1942).

Rate of reactions is dependent on temperature, presence of inhibitors or catalysts, and nature of the substrates, the amount and degree of unsaturation of the component fatty acids

(Hudson, 1983; Min and Boff, 2002); the unsaturated acids are oxidised at different rates, e.g. linoleic acid is oxidised 64 times faster than oleic acid (Hamilton, 1983).

According to Galliard (1983), free radical reactions do not occur in intact dry seeds, because of endogenous antioxidants and low water activity. Oxidation occurs in derived product or after long lasting oxidation of membrane lipid and parallel reduction of natural antioxidant. Autoxidation in complex materials such as flour and its derivatives can cause chemical and physical changes and thus changes in functional properties (Galliard, 1983).

The first phase, induction period, starts with the abstraction of a hydrogen atom adjacent to a double bond in a fatty acid resulting in alkyl free radical (Chan, 1987) by mechanisms not fully understood (Hamilton, 2005). The initiation process can be divided into two substrate types: (RH) substrates (1) and (ROOH) substrate (2, 3); in context with activating energy, most important process is (3) resulting in peroxyl radical (Chan, 1987).

$$RH \rightarrow R^{\bullet} + {}^{\bullet}H \quad (1)$$

$$ROOH + \rightarrow RO^{\bullet} + {}^{\bullet}OH \quad (2)$$

$$2ROOH \rightarrow ROH + H_2O + ROO^{\bullet} \quad (3)$$

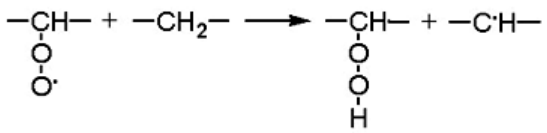
The direct reaction of a lipid molecule with a molecule of oxygen is highly improbable; oxygen has to be activated (formation of singlet oxygen, hydrogen peroxide, superoxide anion, hydroxyl radical or active oxygen-iron complex) by some type of oxidative initiators, such as chemical oxidizers, transition metals, enzymes (Erikson, 2002) or irradiation (chapter photo-oxidation). Detail study of oxidative initiators was investigated by Bulkley (2002).

In the first phase, induction period, the reactions go slowly and at a relatively uniform rate (Hamilton, 1983) and the changes are hardly detectable (Hudson, 1983).

In the second phase, the propagation, the resultant radical react with oxygen to form an unstable peroxy free radical (1). This radical forms a hydroperoxide (ROOH) and new radical by abstracting hydrogen from another fatty acid (Shahidi and Wanasundara, 2002) (2). The produced hydroperoxides are the primary oxidation products; they are generally unstable and quickly decompose to the secondary oxidation products (DeMan, 1992).

The reaction provides a further free radical, which has a rapidly accelerating rate of oxidation and making it a self-propagating chain process (Hamilton 1983; Hudson, 1983). The oxygenation reaction has almost zero activation energy (Chan, 1987); this phase is characterized by rapid absorption of oxygen (Hudson, 1983).

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad (1)$$



(2)

The self-propagating sequence can be stopped by termination reactions, in which unreactive compounds are formed by recombination of various free radicals (Hamilton, 1983, Hudson, 1983). In the termination phase of oxidation, relatively unreactive compounds are formed including mainly aldehydes, ketones and hydrocarbons.

$$R^{\bullet} + {}^{\bullet}R \rightarrow RR$$

$$R^{\bullet} + ROO^{\bullet} \rightarrow ROOR$$

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$$

Photooxidation

Photo-oxidation is an alternative to the free radical mechanism but with different resulting hydroperoxides. Light-induced lipid oxidation depends on the absence or presence of photosensitizers (e.g. chlorophyll, porphyrins, myoglobin) (Hamilton, 1983). These substances, which became excited after absorbing visible light or UV radiation, can induce oxidation in two different ways. The reactions based on free radical mechanisms are induced through Type I, or singlet oxygen is produced in Type II; both types of process can occur simultaneously (Wold, 2006). Singlet oxygen produced from O_2 by light in the presence of photosensitizer is highly electrophilic, and it reacts rapidly with unsaturated lipids; the reaction can be 30 000 times quicker (oleic acid) than autoxidation (Frankel *et al*., 1979).

$$^3Sens^* + {}^3O_2 \rightarrow {}^1O_2 + {}^1Sens \quad \text{(Type I)}$$

$$^1O_2 + RH \rightarrow ROOH \quad \text{(Type II)}$$

Lipoxygenase

The lipoxygenase (so-called LOX) refers to a group of enzymes that catalyses reaction between oxygen and polyunsaturated fatty acids containing methylene interrupted bonds; lipoxygenase isoenzymes can react with triglycerides (Hamilton, 1983). The enzyme is widely distributed throughout the plant kingdom (Hildebrand, 1989), in various tissues (Zhuang and Barth, 2002).

Cereal grains, as other plant tissues, contain the lipoxygenases that are capable of catalyzing the direct oxidation of lipids with molecular oxygen. The enzymes have very low

activity on the fatty acid portions of other lipids and are active only on polyunsaturated fatty acids. The processes usually do not occur in disrupted plant cells, but may be catalyse by co-oxidation (formation of hydroperoxide formed a high energy intermediate complex, which is able to initiate other processes) (Galliard, 1983).

The LOX contain one atom of nonheme iron in inactive high-spin state Fe^{II} iron, which is oxidized to Fe^{III} state during catalysis. The enzyme removes hydrogen from the methylene group; the hydrogen abstraction under aerobic conditions results in the free radical (Zhuang and Barth, 2002).

1.4.3. Hydroperoxide breakdown products

The initial product of lipoxygenase action, a fatty acid hydroperoxide, is stable only under favourable conditions (low temperature, dilute solution, the presence of antioxidants and the absence of catalyst, Gardner, 1987), but normally is very unstable and breaks down by subsequent reactions into the particularly volatile aldehydes and ketones, as well as other breakdown products (Rossel, 1983). The conversion of the hydroperoxides into the final components is less understood than the production of hydroperoxides (Hamilton, 1983); the lack of knowledge is caused by difficult analysis of volatile oxidation products and presence of unstable monohydroperoxides, which can easily degrade (Grosh, 1987).

The spontaneous dissociation of ROO-H requires high activating energy of 90 $kcal.mol^{-1}$ (Gardner, 1987). The reaction is initiated by previously formed radical or in the presence of a catalyst, especially transition metals, which can be introduced into the product from processing equipment (Hamilton, 2005). The activation energy of the cleavage (β-cleavage) of RO-OH is lower (44 kcal/mol) (Min and Boff, 2002), thus the decomposition of hydroperoxides involves forming of a alkoxy and hydroxyl free radicals, which later on form other substances (DeMan, 1992).

$$R–CH(OOH)–R \rightarrow R–CHO^{\bullet}–R + {}^{\bullet}OH$$

Many secondary products of rancidity can be toxic (Chow, 1992; Quackenbush, 1945). The most common symptoms are e.g. diarrhoea, poor rate of growth, cardiomyopathy, haemolytic anaemia, respiratory problems, muscular dystrophy etc. The most important toxic substances can be divided into three distinct classes: peroxidised fatty acids and their end-products, polymeric substances and oxidized sterols (Sanders, 1983).

Aldehydes

Aldehydes are the most important volatile compound produced during decomposition of hydroperoxides; they are more stable and diffusible than hydroperoxides (Enoiu *et al*., 2000). Produced aldehydes depend on the either side of the carbon containing the oxygen atom, thus there are many aldehydes which can be produced (Hamilton, 1983). Saturated aldehydes can contribute to bitter flavour and adverse odour; sensory properties of the most common saturated aldehydes are described as sharp-irritating (C3), bitter-almond (C5 and C6), soapy (C7), and fatty (C8) (Grosch, 1987). Unsaturated aldehydes are not usually connected with off-flavours, e.g. 2,4-dienals are noted as sweet, the dienals aldehydes with chain lengths from C_8 to C_{12} are reported to make a positive contribution to the flavour of chocolate (Hamilton, 2005). Aldehydes are very reactive and can react with proteins (Chopin *et al*., 2007; Comporti, 1993; Kwon *et al*., 1965; Roubal, 1971); the reactions change functional properties of cereal products (Galliard, 1983).

The major products of linoleic acid decomposition, which is the major fatty acid both in proso millet and oats, are 2-hydroxyheptanal (Lehtinenen *et al*., 2003; Loidl-Stahlhofen and Spiteller, 1994), hexanal, and pentanal (Heiniö *et al*., 2002; Lehto *et al*., 2003; Sjövall *et al*., 1997). Other reported aldehydes originated in oat rancidity are heptanal, oktanal, nonanal (Molteberg *et al*., 1996), (*E*)-2,3-epoxyoctanal (Sjövall *et al*., 1997), methylpropanal and methylbutanal (Heinö *et al*., 2002).

Other off-flavours

Aldehydes can pick up an ($OH^{\bullet}$) radical to form the alcohol or an ($H^{\bullet}$) radical to form a hydrocarbon. Alcohols contribute to the flavour in the same way as the aldehydes, but in milder way. They prove range from grassy (C5), solvent to grassy (C7), blue cheesy. The flavour of aliphatic acid range form C_2 vinegary, C_3 sour to C_8 goat cheesy; C_{14}-C_{18} have with very little odour. Ketones contribute a piercing sweet fruitiness, from C_3 sweet, to C_{11}, fatty, sweet (Hamilton, 1983). During oat rancidity, 1-pentanol, 1-hexanol, 2-ethylfuran, 2-pentyl-furan (Heinö *et al*., 2002), 2-heptanon, 1-okten-3-ol (Molteberg *et al*., 1996), and methylethylbenzene (Sjövall *et al*., 1997) were reported. The most adverse effect on sensory attributes has according to Molteberg *et al.* (1996) 2-pentyl-furan, 1-hexanol, and 2-heptanon.

1.4.4. Factors affecting rancidity

There are many factors, which can influence formation and development of rancidity. Generally, these factors include temperature, water activity, nature of the substrates, concentrations of enzyme, and substrate(s) and the presence of inhibitors or catalysts (Galliard, 1983). Understanding the processes and adequate technological intervention can solve many problems of food oxidation or at least kept them under control (Löliger, 1983).

Temperature during storage or processing affects development of rancidity. Heat treatment influences the storage life due to deactivation or increasing activity of enzymes (Gates *et al*., 2004; Keeling *et al*., 1993; Malekian *et al*., 2000) and natural antioxidants (Hamilton, 1983; Lehtinen *et al*., 2003). Storage temperature can influence formation of toxic compounds during lipid deterioration (Sanders, 1983). Higher rate of temperature leads to higher level of rancidity reactions, which is employed in some types of accelerated heating tests. Although there are many studies of influence temperature on meat, milk, and other foodstuffs, only few of them are pointed to cereals. In general, increasing temperature above the ambient temperature, which is specific for each product, leads to increasing of rancidity and decreasing of a storage-life. No significant changes in nutritional quality was observed during storage at 10°C of wheat grains (Rehman and Shah, 1999), maize grains (Rehman *et al*., 2002), rice (Ramezanzadeh *et al*., 1999), proso millets (Al-Kahtani, 1985), and oats (Heiniö *et al*., 2002; Larsen *et al*., 2005; Molteberg *et al*., 1996; White *et al*., 1999).

Water activity (A_w) in food system has great impact on food stability (more than total water content). Water serves as solvent and also interacts chemically (hydrogen binding); water can decrease activity of the metal catalyst (Labuza *et al*., 1971), or can bond with hydroperoxides (Rahman and Labuza, 2007). Water activity can increase the oxidation and sensory changes, as was described on some oil-yielding crops (Reed *et al*., 2002; Kinderlerer and Hatton, 1991) or dried meat (Quaglia, 1988). Inhibitory effect of water is most pronounced in the initial stages of oxidation (Maloney *et al*., 1966). Microbial stability is influenced by water activity (Abdullah *et al*., 2000; Abellana *et al*., 1999; Abellana *et al*., 2001; Gibson *et al*., 1994; Ramos *et al*., 1998; Valík *et al*., 1999). Detail review of water activity in foods was treated by Mathlouthi (2001).

Antioxidants are substances capable to prevent or slow oxidation of other molecules, or can reduce the amount of free radicals; their role is to maintain food quality, extend shelf life and reduce nutritional losses (Reische *et al*., 2002). These substances can be present as

natural constituent, or be purposely added during processing. Antioxidants are very effective on autoxidation, but less on enzyme oxidation (Galliard, 1983). Antioxidants naturally occurring in grains are biologically active compounds such as tocopherols, L-ascorbic acid, thiols, phenolic amino acids and phenol compounds. Phenolic compounds have a key role in lipid oxidation by inhibiting lipase activity (Zadernowski *et al*., 1999). External antioxidants can be divided according to their origin into natural and synthetic. In recent time, there is a trend toward using the natural ones (Hamilton, 2005); many of them are found in spices and herbs (Aguirrezabal *et al*., 2000; Bishov *et al*., 1977) and in vegetable oils (Baldioli *et al*., 1996). Effects of antioxidants on food quality were summarized by Frankel (1996), Valenzuela and Nieto (1996), and Adegoke *et al*. (1998).

Pro-oxidants are substance which can increase the susceptibility to oxidation through creating free radicals or inhibiting antioxidants (Korycka-Dahl and Richardson, 1980**)**. In this group of substances, the carotenoids (Vara-ubol and Bowers, 2001) and chlorophyll (Abraham and DeMan, 1986) are the most common natural products which act on fats (Deuel, 1957). Some metal ions can promote rancidity (Exley, 2004; Maiorino *et al*., 1993; Mercuse and Fredriksson, 1971).

Development of rancidity reactions is greatly dependent on the degree of unsaturation (Visioli *et al*., 1998), e.g. the rate of the series of C_{18} fatty acids (18:0, 18:1, 18:2, 18:3) is 1:100:1200:2500, respectively (DeMan, 1992). Unsaturated fatty acids are more susceptible to oxidation due to the lowered activation energy in the initiation phase (Min and Boff, 2002). The most common unsaturated acids are oxidised at different rates (Visioli *et al*., 1998; Hamilton, 1983), e.g. linoleic acid is oxidised 64 times faster than oleic acid, and linolenic acid 100 times faster than oleic acid (Hamilton, 1983). Approximately 68 % of linoleic acid is oxidized during 3 hours of incubation in AAPH (2,2'-azobis(2-amidinopropane) hydrochloride), in contrast to only 13 % of oleic and 2 % of stearic acid. (Visioli *et al*., 1998). Oleic acid is less susceptible to oxidation (Molteberg *et al*., 1995).

1.4.5. Methods for rancidity evaluation

Rancidity, as a qualitative term, is quantifiable with difficulties; rancid taste is dependent of subjective human threshold for detecting off-flavours (Hamilton and Kirstein, 2003). For detection, measurement and evaluation of lipid deterioration, there are many experimental methods, ranging from organoleptic evaluation to chemical and physical methods (Shahidi and Wanasundara, 2002). Because of the complex and interconnected

changes resulting into a wide range of intermediates and end products, there is no ideal or standard method for covering all changes occurring in the system. Majority of the methods is based on measuring products of oxidative rancidity, because primarily these substances comprise off-flavours and can be monitored by chemical analysis (Hudson, 1983). Methods are generally divided into three groups – measuring primary changes, measuring secondary changes and determination of the resistance to these changes (Rossel, 1983).

Primary changes are evaluated by detection of chemical changes in reactants, especially in fatty acids. Formation of hydroperoxides and peroxides during initial stages of rancidity is monitored by Peroxide value (PV), reported as milliequivalents of formed peroxide per kg of fat. The titratable acidity, which refers to FA liberation, can be evaluated by the direct titration. Thanks to its simple and cheap implementation, this method is widely used by producers to control the quality of their products (Smith, 2002; Shahidi and Wanasundara, 2002).

The primary oxidation products are intermediates that decompose into various secondary products. The TBA (thiobarbituric acid value) spectrometrically detects a pink malonaldehyde-thiobarbituric acid complex (absorption maximum at 530-532 nm), produced by reaction of two molecules of TBA reagents and a malonaldehyde molecule (Mercuse and Johansson, 1973; Koning and Silk, 1963). Anisidine Value (p-AnV) is based on the spectrometric detection of a yellowish complex formed from p-anisidine reagent and non-volatile aldehydes (Tompkins and Perkins, 1999). Connection of P-AnV and PV (2PV + p-AnV) gives Totox value (Shahidi and Wanasundara, 2002). The Oxirane value is based on determining oxirane oxygen by titration with hydrobromic acid, chloride-acetic, or picric acid (Fioriti *et al*., 1966), (in the presence of crystal violet) to a bluish end point (Shahidi and Wanasundara, 2002).

The active oxygen method (AOM), also called Swift Test, is based on measures of PV at different time intervals during bubbling air through the fat under the specific air flow rate. The AOM for the fat is reported as time required to reach 100 milliequivalents (meq) of peroxide per kg of fat (Hamilton and Kirstein, 2003). Commercial apparatus for measuring AOM is known as the Rancimat, and it is very often used for prediction of shelf life of a fat (Shahidi and Wanasundara, 2002).

Secondary products, such as hexanal (Fritsch and Gale, 1977) or pentane (Scholz and Ptak, 1966) can be monitored spectroscopically. NMR (high-resolution nuclear magnetic

resonance) spectroscopy determines detailed characterization of lipid and other minor component and oil stability prediction in one analysis (Hidalgo and Zamora, 2003). Chromatographic methods are widely used for modelling development of fats or oils under different conditions (Shahidi and Wanasundara, 2002). High-performance liquid chromatography (HPLC) can determine aldehydes (Tsaknis *et al*., 1998; Whang and Kim, 2000**)** or triglycerides (Letter, 1993).

Organoleptic properties are the most important sensory determinant of food choice and deteriorative changes lead to unpleasant odours or flavours. Thus sensory evaluation comprises irreplaceable role in the rancidity assessment. The most important evaluated attributes are odour (aroma or fragrance), consistency, texture, and flavour (Meilgaard *et al*., 2006). Analytical tests can be organized as discriminative (difference) tests based on comparison of samples, or descriptive tests based on classification, ranking, rating and scoring (numerical or graphical scale). Affective methods, also called Hedonic tests, measure liking or acceptability of samples. Hedonic tests employ the same methods as analytical tests, but the analytical tests are evaluated by specially trained assessors (Kilcast, 1996). On the basis of sensory evaluation, electronic sensing or electronic nose was developed. This technology can detect and recognize odours and flavours due to reaction of volatile compounds with a specific sensor. This technology has been described in detail by Natale *et al*. (1997), Gardner and Bartlett (1992), and Pearce *et al*. (2006).

2. Objectives

The aim of this thesis was to evaluate development of rancidity in seven selected varieties of proso millets and three selected varieties of common oats.

Partial aims of the thesis:

- Evaluation morphological and phenological characteristics of proso millet varieties.
- Comparison of lipid content and fatty acids composition in oat and proso millet varieties.
- Detection of rancidity development in dependency to different storing conditions and designs in proso millet and oats varieties.
- Recommendation of suitable conditions for storing of high-fat cereals, predispositions characterization of varieties to rancidity.
- Evaluation of rancidity in relation to sensory evaluation.

3. Materials and methods

3.1. Characterization of the experimental trial

The experimental trials were located in the Crop Research Institute (CRI) in Prague, Ruzyne (proso millet), and in the Agricultural Research Institute (VUKROM) in Kromeriz (oats); important characteristics of them are summarized in Table 29.

Table 29: Characteristics of locations of experimental trials.

	CRI	VUKROM
Altitude	338 m above sea level	210 m above sea level
Latitude	50°5´N	49°18´N
Longitude	14°18´E	17°22´E
Production Type	Sugar beet growing region	Sugar beet growing region
Sub-type	Sugar beet-wheat	
Soil texture	Silty-clay	loamy
Soil type	Brown, slightly gleyic	Chernozem, luvi-haplic
Climate	Region moderately dry, mildly warm, mild winter.	Region moderately dry, Warmer with mild winter.

3.2. Materials

3.2.1. Plant material

Nine selected varieties of proso millet, *Panicum miliaceum* (Table 30), were obtained from CRI in 2007 from proso millet collection of Gene Bank, CRI. More than half of the varieties originated in the former Soviet Union, two of them originated in the former Czechoslovakia, and the last one in the China. Varieties were divided into several groups according to experiment. Group A consisted Czech original variety ´Hanacka Mana´, which was employed into optimizing the method. Morphological features were evaluated on six varieties included in group B. Group C comprised of 7 varieties tested on their FFA content and storing changes.

Table 30: Proso millet varieties (EVIGEZ, 2007)

Identificator	Name	Origin	Year of donation	Donor	Group
01Z1100008	´Omskoe 10´	SUN	1992	N.I. Vavilov Research Institute of Plant Industry	B+C
01Z1100019	´Saratovskoe 6´	SUN	1992	N.I. Vavilov Research Institute of Plant Industry	B+C
01Z1100067	´Irtyskoe 201´	SUN	1992	North Central Regional Plant Introduction Station USDA-ARS, Iowa State University	B+C
01Z1100075	´Lung Shu no.14´	China	1992	North Central Regional Plant Introduction Station USDA-ARS, Iowa State University	C
01Z1100143	´Hanacka Mana´	CSK		Czechoslovakia	A
01Z1100144	´Unikum´	CSK		Czechoslovakia	B+C
01Z1100145	´Yantarnoe´	SUN	1996	Ukrainian Research Institute of Plant Production, Genet. and Selection im "Jurijeva"	B+C
01Z1100149	´Gorlinka´	SUN	1996	Ukrainian Research Institute of Plant Production, Genet. and Selection im "Jurijeva"	B+C

Three varieties of naked oats, *Avena sativa*, var. *inermis* (Table 31) were obtained from VUKROM in 2006. All varieties originate in the former of Czechoslovakia or in the Czech Republic. Variety ´Abel´ was utilized for optimizing the method, all three in the final experiment and sensory evaluation.

Table 31: Oat varieties (EVIGEZ, 2007)

Identificator	Name	Origin
03C0701716	´Abel´	Czechoslovakia
03C0702038	´Saul´	Czech Republic
03C0701947	´Izak´	Czech Republic

3.2.2. Sample preparation

Before the experiments started, three processing designs were applied: (1) whole grains, (2) ground whole grains (particle size 0.8 mm, prepared on Cyclotec 1093 Sample mill) and (3) flour (particle size 0.25 mm, prepared on Brabender). All prepared samples were stored in paper bags in two storing conditions: (1) laboratory condition (average temperature 23.4°C, relative humidity 42.3 %) and (2) freezer (average temperature 1-2 %, relative humidity 70 %). During experiment data of moisture and temperatures of laboratory storage conditions were recorded (Figure A1 in Appendices). For optimizing the method, only ground whole grains were stored in laboratory conditions. For sensory evaluation, oat flakes made on manual flaker mill from whole stored grains were applied.

3.3. Establishment of the field trials

All proso millet plants were manually sown from original seeds from the Gene Bank CRI. There was established one trial plot of 5 m^2 for each variety. The spacing between rows of seeds was 12.5 cm. The sowing dates were 6th May in 2006 and 11th May in 2007 according to the optimal climatic condition. The main operation during the vegetation period was the weed reduction. The morphological features and growth stage were observed and evaluated.

Experimental trials for oat varieties were established according to the standard method of VUKROM.

3.4. Evaluation of morphological features and growth stages

The classification methodology used for evaluation of proso millet was the international Descriptors for *Panicum miliaceum* and *P. sumatrense* (IBPGR, 1985). Ten randomly selected plants of the same variety were evaluated for each morphological trait corresponds. During the vegetation period were phonological phases measured. As the day of the emergency is considered the day, when the primary leaves appear over the soil surface and form a visible row. As the first flowering day is rated the day when 50 % of plants have completely open inflorescences. The ripeness starts when 75 % of the achenes are ripened. The examinated features are summarized in Table 32.

Table 32: Classificated features.

Features	Definition or classification
Plant height	Measures (cm) from ground level to tip of inflorescence; in case of decumbent or prostrate plants, length of flowering culm from rooted base.
Fruit colour	1 yellow 4 red 7 reddish brown
Fruit	The 1000 kernel weight (g).

3.5. Optimization of method

Before the experiment started, the optimal method has been tested. All methods were based on Czech state norm CSN 56 0512-9 – ´Determination of titratable acids´, originally designed for wheat and rye. This method consisted in the simple titration of a flour suspension or a filtrated suspension with a hydroxide (0.1 M NaOH or 0.1 M KOH) with phenolphthalein

as an indicator of the end point. Besides these two methods, another modificated method with 60 % ethanol as a solvent of samples was tested.

Grains were prepared by milling before test exactly. Little amount of interspersed sample was milled and then removed. Than approximately 50 g of sample was milled; 90 % of the particles should be less then 1mm. The milled sample was mixed carefully.

For the direct titration the suspension, 10 g of prepared flour of a sample was dissolved in 100 ml of distilled water; initially 20 ml of distilled water was added, thoroughly mixed and then the rest was added. After 30 minutes of stirring the sample, about 5 drops of phenolpthalein were added and then the sample was titrated with NaOH solution, until the rich pink colour resisted for 1 minute.

For titration the filtrated suspension, a sample was dissolved in 200 ml of distilled water, and after 30 minutes of stirring, suspension was filtrated through the folded filter paper. Initially 20 ml were removed and from the rest filtration, 50 ml were transferred and titrated as described before.

Final titratable acidity (*A* for direct titration or *Y* for titration of filtrated suspension), expressed as consumption of NaOH (in mg) per 100 g of dry matter of a sample, was calculated:

Direct titration

$$A = \frac{1000 \times V \times c}{m} \times \frac{100}{100 - w}$$

Titration of filtrated suspension

$$Y = \frac{4000 \times V \times c}{m} \times \frac{100}{100 - w}$$

V	consumption of NaOH [ml]
c	NaOH concentration [mol.l^{-1}]
w	water content in the sample [%]
m	sample weight [g]

For optimizing the method, oat variety ´Abel´ and proso millet variety ´Hanacka Mana´ were employed. They were stored as whole grains in laboratory conditions and tested in week intervals for 5 weeks.

3.6. Fatty acids analysis

The content of petrolether (4-hydroxy-3,5-dimethoxybenzaldehyde) extractable lipids was determined by Soxhlet extraction. Fatty acids were isolated and determined by gas gromatography. Fatty acids were converted the methylesters after lipid acid hydrolysis in HCl

and lipid extraction with chloroform ($CHCl_3$). Fatty acid analysis and determination of total fat content were carried out in Food Research Institute, Prague.

3.7. Rancidity analysis

Titratable acidity

For the FFA evaluation, acidity of suspension of samples was determined by a slightly modified titration standard method (CSN 56 0512-9), as described before. During optimizing the method, the samples were tested once a week during one month in three repetitions. Evaluation of storage changes carried out in two weeks intervals during 16 weeks for proso millet in two repetitions for each sample and in monthly intervals during 13 months in 2006 and 2007 for oats in three repetitions for each sample.

Sensory evaluation

Sensory analysis was subjectively evaluated by 10 special trained assessors from Food Research Institute, Prague. Oat flakes were prepared from stored grains by the manual flake-grinder exactly before each test. Five grams of the flakes were mixed with boiling water and the sensory properties of a sample were tested after 15 minutes. The aroma, flavour and the intensity of bitterness were evaluated by simple graphic scale with unsegmented 100 mm long abscissa. The position of a rating mark was transfer into an evaluation scale ranging from 0 to 100 points. Example of form is attached in appendix (Figure A2). Tests were carried out three times during 2006, after 16 and 36 weeks.

3.8. Statistical analysis

Basic statistics were performed by calculation of mean x, standard deviation (s_x) and standard error (s_e). Coefficient of Variation (CV) statistical measure of the dispersion of data points in a data series around the mean. Analysis of variance (ANOVA) and the Tukey HSD test were used for statistical evaluation of differences among tested varieties (software – Statistica 7.0 CZ).

4. Results

4.1. Evaluation of conditions during vegetation periods

Meteorological data were obtained from the station of the CRI, Prague, and the station of VUKROM. Table 33 summarizes the vegetation periods of two experimental years of proso millet cultivation (2006, 2007) in CRI and one experimental year of oats cultivation (2006) in VUKROM. Temperature (°C) refers to monthly average measured at standard 2 m height; the precipitation (mm) is given as monthly sum.

Table 33: Average conditions during vegetation periods.

	Average temperature [°C]	Precipitation sum [mm]
2006 CRI	14.0	414.2
2006 VUKROM	14.2	492.9
2007 CRI	15.4	344.6

In general, the average temperatures were slightly higher in VUKROM Kromeriz, than in CRI Prague, because this area belongs to the warmest regions in Czech Republic. Both years 2006 and 2007 were warmer than the long-term average, by 1°C in 2006 and by 2.4°C in 2007. Average temperatures are showed in Figure A3 in Appendices. The annual precipitation (Figure A4, Appendices) sums were higher in 2006 at both experimental sites, i.e. 104.6 mm and 128.5 mm more than the long-term average, which means over 33 % extra rainfall during the season. In 2007, the precipitation sums were also higher than the long-term average, but not significantly.

In the year 2006, the temperature amplitude was unusually high, 20.7°C in Prague and even 21.1°C in Kromeriz due to cold spring and very hot summer. This year is characterized by colder March, i.e. 2.2°C, 1.3°C and 1.6°C less than the long-term average in Prague and Kromeriz, respectively. April, May and July can be characterized as moderately warm. The average July temperatures were uncommonly warm, i.e. 22.9°C in Prague, 22.7°C in Kromeriz. In late summer during August, average temperatures dropped below the long-term average, as it is illustrated in the graph. Although data from Kromeriz was mostly higher, the maximum difference reached 0.9°C and the development of temperatures during season was comparable to those from Prague. In the year 2007, temperature amplitude was only 13°C, but in general, the average monthly temperatures were higher than long-term average during the whole season, as can be seen in the graph. The spring in 2007 was significantly warmer, i.e.

6.5°C in March and 12.2°C in April, which is 2.7°C and 4.3°C more than the long-term average. May and June were also warmer than long-term average and also warmer then the previous year. The average temperatures reached maximum in July.

The precipitation in the year 2006 was high with uneven distribution with two peaks. The spring precipitation was very high, with maximum reached in May, i.e. 99.0 mm in Prague and 113 mm in Kromeriz. Wet spring and wet summer during the first four months turned into extremely dry July with only 19.6 mm of rainfall in Prague and 9.6 mm in Kromeriz. Then in August, precipitation rapidly increased and reached 99.4 mm and 115.7 mm of rainfall in Prague and Kromeriz, respectively. The year 2007 was still humid? than the long-term average, but not so extraordinary. Extremely dry spring in March and especially April, i.e. only 14.6 mm and 2.4 mm of rainfall, respectively, were followed by excessive precipitation during the rest of the season reaching about 80 mm a month with maximum precipitation in June, i.e. 87 mm.

4.2. Evaluation of morphological traits on proso millet accessions

Evaluation of morphological and phonological traits is summarized in Table A2. The growth period from emergence to maturity showed great differences between experiment years. In the year 2006, the average period was 57 days, and in 2007 it was 76 day, i.e. about 25 % difference. This huge variance is due to difference conditions during growing seasons. Because proso millet is adapted to dry and hot conditions, the year 2006 due to excessive precipitation didn't provide as suitable conditions as in 2007. The period from emergence to flowering was about 26 days with standard deviation (SD) of ± 4.85; the average difference between the years was 4 days. The period from emergence to maturity was (66 ± 12.06) days with great difference between years, i.e. 19 days. The longest period to maturity appeared in variety ´Saratovskoe 6´ and ´Yantarnoe´, i.e. (73 ± 9.90) days and (73 ± 16.97) days. In the year 2007, the significantly low period to maturity (85 days) appeared in variety ´Unikum´. The shortest period to maturity was in variety ´Irtyskoe 201´, i.e. (56 ± 14.85) days. The most unstable and dependent on the condition was variety ´Omskoe 10´, which differed in the duration of period by 31 days. The most stable and independent on the condition was variety ´Gorlinka´, which differed in the duration of period to maturity between years of 10 days.

Plant heights were also greatly affected by the conditions; they ranged from 69 to 73 cm in 2006 and from 84 to 166 cm in 2007. The greatest difference between the years, i.e.

82 cm, appeared in the variety ´Unikum´, which also belongs to the highest plants. On the other hand, very low plants of the variety ´Irtyskoe 201´ differed only 4 cm between 2006 and 2007. The plant height was significant positive correlation with the length of period to maturity (r = 0.71) and with

Yield was greatly affected by the conditions; in 2006, the average yield was almost half in comparison to 2007, i.e. 1429 and 2879 kg/ha. The greatest impact of condition appeared in the variety ´Omskoe 10´, the yield in 2006 was to the lowest and in 2007 was the highest among all. Relatively stable yields were in the varieties ´Unikum´ and ´Gorlinka´, which belonged to the lowest yielded varieties. Yield was in positive correlation with days to maturity (r = 0.38).

Grain colour, which is not included in Table A2, was predominantly reddish brown; only variety ´Unikum´ had red grains.

Weight of thousand seeds (WTS) was relatively stable feature, the average WTS in 2006 and 2007 differed only in 0.2 g. But great variance among varieties appeared; the highest results showed the variety ´Gorlinka´ and the lowest were found in the variety ´Unikum´, i.e. (7.7 ± 0.19) g and (5.8 ± 0.33) g, respectively. WTS was significantly negative related with plant height (r = -0.72).

Fat content demonstrated great influence of condition, variance of varieties was low. Fat content tends to be in negative correlation with WTS. Highest fat content was in the variety ´Irtyskoe 201´ and the lowest in the variety ´Yantarnoe´, i.e. (4.57 ± 0.52) and (4.15 ± 0.26) g/100g in dry matter. Variety ´Yantarnoe´ was very stable in fat content. Total lipid content was negatively related to the WTS (r = -0.39) and days to maturity (r = -0.35).

4.3. Fatty acid content

Data for the average fatty acid composition of three oats and seven proso millet varieties are demonstrated in Figure 8 and summarized in Table A1. In this experiment 13 different fatty acids in oats and 12 different acids in proso millet were detected. Erucic acid (22:1) was excluded from the evaluation of proso millet varieties, because it was not detectable in varieties ´Lung Shu no. 14´, ´Yantarnoe´, and ´Gorlinka´.

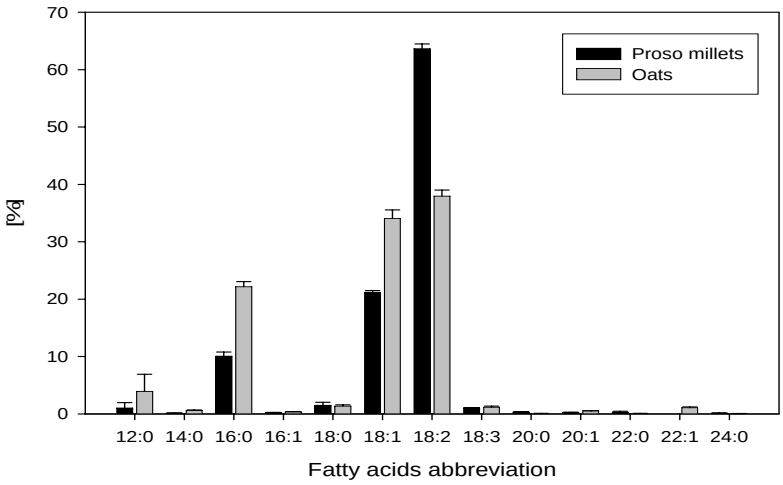


Figure 8: Average fatty acid composition in oat and proso millet varieties.

Although three predominant fatty acids (linoleic, oleic, and palmitic) together accounted about 95 % in both proso millet and oats, their proportion, and also proportion of unsaturated and saturated fatty acids differed. Unsaturated fatty acids comprised 86.4 % in proso millets and 75.2 % in oats. Linoleic acid was predominant in both proso millets and oats, but in proso millet accounted for almost two thirds, (about 64 %), and only about 38 % in oats. Oleic, palmitic, and lauric acid in oat grains were founded in higher amounts then in proso millet, i.e. about 34, 22, and 4 % in oats and 21, 10, and 1 % in proso millets. Other fatty acids were found in minute amounts. Lauric acid content greatly varied in both proso and oat varieties.

Oats lipids comprised (5.85 ± 0.65) g/100g of dry matter, on average. ´Abel´ demonstrated the highest fat content (6.29 %) and slightly higher content of unsaturated acids, i.e. 76.0 %. ´Saul´, with the lowest fat content (5.1 %), demonstrated slightly lower unsaturated fatty acid level, i.e. 74.4 %. Lipid content was found to be positively related ($r = 0.88$) with the total content of unsaturated fatty acids.

Fat content in proso millet varieties ranged from 3.9 % in ´Lung Shu no. 14´ to 4.5 % in ´Irtyskoe 201´; average content of proso millets was (4.19 ± 0.23) g/100g of dry matter. Slightly higher content of unsaturated fatty acids was in ´Irtyskoe 201´ (87.7 %) and ´Unikum´ (87.6 %); slightly lower content was in ´Gorlinka´ (85.7 %). Total contents of unsaturated fatty acids were positively related ($r = 0.47$) to the total fat content. Lipid content was positively related to the proportion of linoleic ($r = 0.45$), linolenic ($r = 0.48$), and

gadoleic ($r = 0.34$) acids and negatively related with the proportion of myristic ($r = -0.69$), stearic ($r = -0.34$), and arachic ($r = -0.35$) acids.

4.4. Optimizing of the evaluation method

For our experiment the method of direct titration of distilled water suspension was chosen with a slight modification, because of suspensions non transparency and discoloured. So the modification for our purposes includes using a reference electrode instead of phenolphthalein as an indicator of the end point. The endpoint pH 9.5 was determined on the basis of repeated blank experiments with phenolphthalein as the indicator.

4.5. Evaluation of rancidity in proso millet

The titratable acidity, measured by titration among seven proso millet varieties, three processing designs and two storing conditions is summarized in Tables 34 and A3 (Appendices). The results demonstrated impact of all factors. In general, the greatest differences were among processing designs; very rapid development of titratable acidity appeared on groats, storing of flour and whole grains tend to be lesser affected. The influence of storing conditions was dependent on processing design; grains demonstrated only minute and flour only little dependence on storing conditions, whilst stored groats are affected a lot.

Table 34: Average titratable acidity of proso millet varieties (mean values ± SD).

		Flour	Groats	Whole grain
		[mg of NaOH/100g of dry matter]		
Storage conditions	Freezer	53.2 ± 7.3^{a}	112.4 ± 23.7	54.7 ± 5.1^{a}
	Laboratory	59.0 ± 9.6^{b}	123.0 ± 24.6	54.3 ± 4.6^{b}
Variety	01Z1100008	50.5 ± 6.0^{b}	108.6 ± 25.6^{b}	50.0 ± 3.5^{b}
	01Z1100144	56.1 ± 9.9^{a}	123.3 ± 25.1^{a}	52.9 ± 3.2^{a}
	01Z1100019	54.5 ± 6.3^{a}	119.7 ± 22.4^{a}	53.8 ± 4.0^{a}
	01Z1100067	54.8 ± 9.2^{a}	104.0 ± 19.7^{b}	53.5 ± 4.6^{a}
	01Z1100075	59.6 ± 9.6^{c}	121.7 ± 25.1^{a}	57.3 ± 4.2^{d}
	01Z1100145	54.7 ± 7.7^{a}	123.4 ± 25.8^{a}	55.0 ± 4.2^{c}
	01Z1100149	62.3 ± 9.0^{d}	123.1 ± 22.3^{a}	59.1 ± 4.5^{e}
Average		56.1 ± 9.0	117.7 ± 24.6	54.5 ± 4.87

Values of parameters marked by same indexes are not significantly different at $p \leq 0.05$.

Processing design had the greatest impact on the development of titratable acidity during storage period and also influenced response to storing conditions. The average titratable acidity of flour, groats, and whole grains during the whole period of storage, was

approximately 56.1, 117.7 and 54.5 mg of NaOH/100g of dry matter. The 16 weeks storage increased the acidity by approximately 22.4, 70.8, and 11.4 mg of NaOH/100g of dry matter for the processing designs, flour, groats, and whole grains, respectively.

The titratable acidity of flour was the lowest at the beginning of the experiment, i.e. 42.7 and increased to 65.1 mg of NaOH/100g of dry matter at the end. The most significant changes in titratable acidity appeared during first two weeks in laboratory storing conditions, and after that the increase slowed downward. During storing in the freezer, the most noticeable changes appeared in 6^{th} to 10^{th} weeks. After 16 weeks of storage, average flour titratable acidity was only little higher than the titratable acidity of stored whole grains; the titratable acidity of flour stored in freezer was even lower than for whole grains. But because the initially titratable acidity was different, the increase of titratable acidity was almost double in flour than in the whole grains, i.e. the increase by 22.4 for flour 11.4 mg of NaOH/100g of dry matter for grains. Remarkably stable flour with little increase of titratable acidity in comparison to the rest appeared in flour of variety ´Omskoe 10´, especially in freezer, i.e. the increase by 22.5 in laboratory and only by 8.0 mg of NaOH/100g of dry matter in freezer storing conditions.

The stored groats was the most affected processing design; the titratable acidity doubled during 16 weeks of storing. The titratable acidity was the highest both at the beginning (74.4) and the end (145.2 mg of NaOH/100g of dry matter) of the experiment. Extremely rapid development of titratable acidity appeared during the first two weeks in laboratory storing conditions; in comparison to flour, these changes were almost 3.5 times higher. From second weeks to tenth weeks, the increase of titratable acidity slightly dropped; after 14 weeks, the changes suddenly dropped. Appearance of the significant increase of titratable acidity arised later in freezer, usually after 6 to 10 weeks, and than the changes slowly decreased. The groats from the variety ´Irtyskoe 201´ were relative stabile in comparison to others and demonstrated the lowest changes both in freezer (increase by 51.0) and laboratory conditions (increase by 67.1 mg of NaOH/100g of dry matter). On the other hand, variety ´Gorlinka´ showed the greatest changes, i.e. increase by 84.8 and 72.5 mg of NaOH/100g of dry matter in laboratory conditions and freezer, respectively.

Stored grains changed only a little during storing for 16 weeks and they were not affected by storing conditions at all. The average titratable acidity was 48.8 at the beginning and 60.2 mg of NaOH/100g of dry matter at the end of the storage period. The development

of changes was very similar in freezer and laboratory storing conditions and the average titratable acidity differ minimally, i.e. 54.7 and 54.3 mg of NaOH/100g of dry matter in freezer and laboratory conditions, respectively. The increase of titratable acidity arised gradually, the most significant changes appeared between 4th and 6th weeks, but these changes were still only minute. Great storing stability showed the variety ´Unikum´; the increase was only 6.2 in laboratory conditions and 10.2 mg of NaOH/100g of dry matter in freezer.

Storing conditions, as has been mentioned before, was affected by processing design and have no significant impact on stored whole grains. In general, storing conditions greatly affected groats; the average titratable acidity in laboratory conditions was 123.0 and in freezer 112.4 mg of NaOH/100g of dry matter. Flour and grains was influenced only little, the difference between laboratory conditions and freezer was 5.8 for flour and only 0.4 mg of NaOH/100g of dry matter for stored whole grains. Storing conditions greatly influenced the progress of changes in groats; the increase of titratable acidity during the first 14 days reached 38.8 in laboratory conditions and 3.4 mg of NaOH/100 g of dry matter in freezer. In laboratory conditions, the gain decreased slowly during following conditions, whilst in freezer increased until 14th week. Different results under different storing conditions appeared in variety ´Lung Shu no. 14´ and ´Irtyskoe 201´; the titratable acidity of ´Lung Shu no. 14´ was relatively higher than the average in laboratory conditions and lower than the average in freezer, variety ´Irtyskoe 201´ had reversely results.

The titratable acidity was little affected by variety. The lowest average titratable acidity was for variety ´Omskoe 10´ (69.7 ± 36.0 mg of NaOH/100 g of dry matter). Variety ´Gorlinka´ reached the highest average titratable acidity, i.e. (81.5 ± 32.7) mg of NaOH/100 g of dry matter; it had highest acidity of grains and flour. The highest titratable acidity of groats was found in variety ´Irtyskoe 201´, i.e. (104.2 ± 19.7) mg of NaOH/100 g of dry matter. There was found positive correlation of average titratable acidity with total content of unsaturated acids (r = 0.43), especially linoleic acid (r = 0.42). No correlation was found with total fat content.

4.6. Evaluation of rancidity in oats, long-term storage

Titratable acidity

Average titratable acidity of three oat varieties during 13 months of storing in two different storing conditions is presented in Table 35. Results from measure after 1 month are excluded from the evaluation as a consequence of fault pH meter.

Table 35: Average titratable acidity of oat varieties (mean values ± SD).

	´Abel´	´Izak´	´Saul´
	[mg of NaOH/100g of dry matter]		
Freezer	87.3 ± 1.1[a]	77.7 ± 1.2[a]	71.8 ± 0.8[a]
Laboratory c.	91.6 ± 2.1[b]	85.7 ± 1.7[b]	74.3 ± 0.8[b]
Average	89.4 ± 1.3	81.7 ± 0.9	73.0 ± 0.5

Values of parameters marked by same indexes are not significantly different at p≤0.05.

Average titratable acidity was significantly different ($P < 0.05$) between storing condition, i.e. (78.9 ± 6.5) in freezer and (83.9 ± 7.4) mg of NaOH/100g of dry matter in laboratory conditions. The greatest difference between storing conditions was found in variety ´Izak´, ex. difference about 7.7 mg of NaOH/100g of dry matter. Titratable acidity of variety ´Izak´ significantly increased in laboratory conditions.

Differences among varieties were significant ($P < 0.05$). Variety ´Abel´ demonstrated the highest average titratable acidity. Although the titratable acidity was the highest both at the beginning and at the end of the experiment, during the storage period, titratable acidity increased only by 1.1 in freezer and even decrease by 0.7 mg of NaOH/100g of dry matter in laboratory conditions. Although variety ´Izak´ didn't change in freezer, its titratable acidity increased from 81.7 to 86.5 mg of NaOH/100g of dry matte in laboratory conditions. Variety ´Saul´ had the lowest average of titratable acidity; titratable acidity was the lowest both at the beginning and at the end of the storage period. ´Saul´ demonstrated very little differences between storing conditions and low increase of titratable acidity, i.e. by 1.1 in freezer and 0.7 mg of NaOH/100g in laboratory storing conditions. There was found significant positive correlation of average titratable acidity with total fat content ($r = 0.93$) and content of unsaturated acids ($r = 0.99$). Titratable acidity was in significant negative correlation with palmitic ($r = -0.99$) acid.

Sensory evaluation

Sensory evaluation of odour, taste and intensity of bitterness, in three oat varieties is presented in Table 36. Subjective evaluations exhibit great inconsistency among evaluation of individual evaluators, standard deviations of the sensory attributes (s_x) ranged from 8.1 to 23.5. The highest variabilities were observed for intensity of bitterness.

Table 36: Average values of sensory attributes of oat varieties (mean values ± SD).

Variety	Odour [points]		Taste [points]		Intensity of bitterness [points]	
	Freezer	Laboratory c.	Freezer	Laboratory c.	Freezer	Laboratory c.
´Abel´	37.8 ± 15.7	43.5 ± 16.4	43.8 ± 13.6	52.8 ± 15.6	36.7 ± 16.2	50.4 ± 19.6
´Izak´	35.6 ± 15.4	42.4 ± 13.2	46.2 ± 15.2	56.3 ± 13.1	37.0 ± 16.3	55.8 ± 18.6
´Saul´	36.3 ± 15.2	43.6 ± 16.2	43.8 ± 13.6	54.1 ± 15.2	37.8 ± 15.8	51.0 ±21.0
	0 = very pleasant 100 = disgusting		0 = delicious 100 = disgusting		0 = without bitterness 100 = bitterness	

In general, taste spoiled during storage and bitterness intensified during storage period. Odour of samples did not spoil significantly in freezer, but unexpectedly improve in some samples stored in laboratory conditions. Better average sensory properties were obtained from samples stored in freezer than from samples stored in laboratory conditions, i.e. (39.5 ± 6.0) and (49.7 ± 7.0) points, respectively. The sensory evaluation of samples from freezer increased by 3.4 points and by 7.1 points in samples from laboratory conditions. Odour tended to be the best evaluated property, i.e. (40.1 ± 4.9) points; it also demonstrated only little difference (3.9 points) between storing conditions. Evaluation score of odour ranged from 32.9 to 49.6 points. Changes in taste was evaluated as the worst sensory property, its average was (49.5 ± 6.2) points. Taste changed only little during storing in freezer but degraded in laboratory storing conditions, i.e. it reached from 44.8 to 47.4 points in freezer, and from 51.8 to 58.4 points in laboratory conditions. Bitterness intensified a lot during storage period; its average was (44.2 ± 10.1) points. Bitterness increased both in freezer (by 11.4 points) and in laboratory conditions (by 15.7 points). Some samples were described as inedible at the end of experiment.

Variety ´Abel´ demonstrated contradictory evaluation, because it obtained better evaluation of odour at the end of the experiment; samples stored in freezer also improved their taste. Intensity of bitterness of samples stored in freezer changed only little (by 5.9 points); intensity of bitterness of ´Abel´ samples was the lowest both in freezer and laboratory conditions. In general, variety ´Abel´ usually obtained very poor or the worst evaluation at the beginning of the experiment, but mostly the best evaluation at the end.

Variety ´Izak´ reached the worst average evaluation with the highest score. Although its odour obtained good evaluation with slightly improvement of samples stored in laboratory conditions, its taste was evaluated as the worst. ´Izak´ had also the highest intensity of bitterness, i.e. it reached to 46.8 in freezer and even 61.1 points in laboratory conditions. ´Izak´ demonstrated the greatest difference between storing conditions, i.e. 12.3 points on average. Variety ´Saul´ obtained medium evaluation of all sensory properties, its odour also improved in laboratory conditions.

Correlation between titratable acidity and sensory evaluation

Only minute correlation (r = -0.147) was found between average sensory evaluation and development of titratable acidity. Only evaluation of odour was in positive correlation with titratable acidity (r = 0.39). Variety ´Izak´ with medium fat acidity obtained the worst sensory evaluation; it demonstrated greatest difference between storing conditions both in fat acidity and sensory evaluation. Variety ´Saul´ obtained medium sensory evaluation but was of the lowest average fat acidity.

5. Discussion

5.1. Evaluation of morphological traits on proso millet accessions

In comparison to other cereals, proso millet has significantly short vegetation period. Vegetation period ranges from 90 to 110 day in our conditions (Moudrý *et al.*, 2005); period from emergence to flowering ranged from 28 to 50 days in world proso millet germplasm collection (Reddy *et al*., 2007). Relatively early flowering accessions of varieties in this study corresponded to evaluation of varieties from Russia and the former Soviet Union (Reddy *et al*., 2007) and evaluation of variety ´Unikum´ (Moudrý *et al.*, 2005).

The plant height significantly differ among varieties; the average plant height of world proso millet collection ranged from 23 to 105 cm (Reddy *et al*., 2007). Higher varieties evaluated in this study were found to have lower yield. Similar results obtained Channappagoudar *et al.* (2007) in evaluation of nine Indian proso millet varieties.

Various shades of brown colour comprised about 44 % of world proso millet germplasm collection. Yellowish colour was found on about 20 % of world collection. White, red, greenish and black colours accounted for about 17 %, 9 % 8 %, and less than 1 % of world collection, respectively (Reddy et al., 2007).

Russian varieties had significantly higher WTS (about 7 g) in this study than variety ´Unikum´ (5.8 g). Low WTS of variety ´Unikum´ corresponded to relatively lower WTS of varieties from the former Czechoslovakia (Moudrý *et al.*, 2005).

5.2. Fatty acid content

Proso millet and oat contain relatively higher lipid content with high level of unsaturated fatty acids than other cereal grains. It contributes to susceptibility to lipid deterioration, because liberated unsaturated fatty acids are more susceptible to oxidation (Jensen and Risbo, 2007; Min and Boff, 2002) and final product of oxidative reaction caused undesirable off-flavours (Becker, 2002). Despite having lower lipid content, proso millets had significantly higher unsaturated fatty acids (about 87 %), especially linoleic acid (64 %), which is oxidised very fast (Hamilton, 1983). Data of fatty acids composition of proso millet was well in agreement with data previously published in the literature (FAO, 1995; Lorenz and Hwang, 1986). Data of oat FA composition mostly corresponded to other studies (Deane and Commers, 1986; Welsch, 1995; Youngs *et al*., 1977), but in some partial results of FA

composition were different. Moudrý *et al.* (2005) and Welsch (2006) described the differences in the great fluctuation of lipid composition in oats. Three Czech oat varieties used in this experiment showed lower content of unsaturated acids (about 75 %) than founded in Australian collection of oats, reported by Zhou *et al*. (1998a), and in some Norway varieties reported by Molteberg *et al*. (1996).

5.3. Evaluation of rancidity in proso millet

Rancidity of proso millets was evaluated as titratable acidity caused by increasing liberated fatty acids. This method is widely accepted for evaluating rancidity development (Shahidi and Wanasundara, 2002) and was performed in many experiments (Al-Kahtani, 1985; Molteberg *et al*., 1995). This experiment employed slightly modified CSN method (56 0512-9). Seven varieties, three processing designs and two storing different conditions were applied to reveal dependency of rancidity development and possible determining or limiting these processes.

The most remarkable changes occurred during first 2 weeks in groats; in flour and grains, the changes were gradual and not so substantial. Heiniö *et al*. (2002) described the most significant changes in oats during first months of storage. The growth of fat acidity decreased at the end of the experiment, i.e. after 4 months. Al-Kahtani (1985) in experiment with proso millet flour did not found additional changes after 6 months of storing.

Although lipids are precursors of liberated fatty acids, different lipid content among varieties did not correlate to development of titratable acidity. These results corresponded to work of Goffman and Bergman (2003), and Molteberg *et al*. (1995) who reported no influence of lipid content to following development of fat acidity in oats. On the other hand, Sahasrabudhe (1979) demonstrated higher content of FFA in oat varieties with high lipid content. Different correlation among individual fatty acids is in contrast to experiment with oat lipases in pure homoacid triacylglycerols, where oleic and linoleic acids were preferred (Piazza *et al*., 1992). Contrariwise, Heyller *et al*. (1999) demonstrated lipase selectivity to individual fatty acids only in seeds with > 80% saturated acids.

The grain colour had been reported to influence oxidative processes due to different content of pigments with antioxidant properties (Goffman and Bergman, 2004). Although being based on measuring of hydrolytic processes, there was not found correlation between colour and titratable acidity in this experiment. But development of following oxidation

reactions would influence rancidity, especially by production of off-flavours. Generally, dark varieties of cereal grains have higher antioxidant components (tannins and phenolic) (Shahidi and Naczk, 2003). Grains with red brans exhibited significantly higher antiradical activity, demonstrated by Goffman and Bergman (2004) in rice, Shahidi and Naczk (2003) and Siwela *et al*. (2007) in finger millets, by Zong *et al*. (2006) in wheat varieties, and Dykes and Rooney (2006) in sorghums.

The greatest differences of titratable acidity were found among processing designs. Only minute changes appeared in stored whole grains. These results are in accordance to widely accepted opinion, that grains are intact, relatively stable and can be store for a long time (Molteberg *et al.*, 1996; Mills, 1996). The experiments of Hansen and Rose (1995) with wheat; and experiments of Ekstrand *et al*. (1992) with oats, described the lowest changes of FFA occurred in stored whole wheat grains. Although flour comprised of the smallest particles, titratable acidity of stored flour did not increase so significantly as stored groats. Titratable acidity of stored groats almost doubled during 16 weeks. In spite of this result Kaur *et al*. (1993) supposed particle size to be important factor affecting lipase activity. Although groats were of greater particles, groats from whole grains contained brans. Lipase enzymes, which cause liberating of FFA, are located mainly in these outer layers (Martin and Peers, 1953; White, 1999). Thus, groats could be more susceptible to rancidity reactions due to higher rates of lipolysis.

Storing conditions influenced development of fat acidity. Samples stored in laboratory conditions (about 23°C) deteriorated significantly more than those stored in freezer (1-2°C). It confirmed the findings of Rehman (2006) in wheat, maize, and rice, Rehman and Shah (1999) in wheat, Ramezanzadeh *et al*. (1999) in rice bran, and experiment of (Al-Kahtani, 1985) with proso millet flour. In all experiments, only minute changes occurred on cereals stored at lower temperature than 10°C and significant changes occurred at temperature over 25°C. Also Hansen and Rose (1996) concluded in their study, that ordinary room temperature contributes to development of rancidity. Lipases have optimal temperature over 30°C (Rose and Pike, 2006; Lee and Hammond, 1990; Sahasrabudhe, 1982), so temperatures approaching this value during of storing conditions can lead to higher activity of these enzymes.

5.4. Evaluation of rancidity in oats, long-term storage

Development of rancidity of long-term storage of three Czech oat varieties was evaluated with the same method, as in previous experiment with proso millets. No significant changes in titratable acidity were found in samples stored in freezer, and only slight increase of titratable acidity was found in samples stored in laboratory conditions. As mentioned before, grains are considered to be relatively stable and intact, and thus can be store for a long time (Mills, 1996; Molteberg *et al.*, 1996).

Difference of titratable acidity among oat varieties, could be caused by their different lipid content. There was found very strong correlation between fat content and titratable acidity. Goffman and Bergman (2003) published very similar results in rice. Significant correlation between fat acidity and content of unsaturated fatty acids corresponded to lipase selectivity to unsaturated fatty acids demonstrated by Piazza *et al*., (1992) in oats and by Sagiroglu and Arabaci (2005) in sunflower. Molteberg *et al*. (1996) and Peterson (2001) concluded also the influence of presence/absence of compounds with antioxidant properties.

Being highly subjective, sensory evaluation can demonstrate inconsistency, contradictory evaluations and high variability (Min and Kim, 1985). This fact can explain the high variability among individual assessors in this experiment. The major difference in sensory attributes was found between storing conditions; all three evaluated attributes of samples stored in freezer were described as more pleasant and with significantly lower intensity of bitterness. These findings are in agreement to lower titratable acidity of samples form freezer. Same correlation between fat acidity and sensory evaluation during long-term storage were described in variety ´Veli´ by Heiniö *et al*. (2002). Despite only low changes of fat acidity, sensory attributes degrade more significantly. The discrepancy between minute changes in fat acidity and significant change in sensory attributes could be explained by very low thresholds of off-favours (Frankel, 1985; Min and Boff, 2002). Thus even very low changes in fat acidity producing minute amounts of compounds with off-favour property could cause very remarkable changes in sensory attributes.

Unexpected minute degradation or even improvement of odour during storage period is in contrast to loss of odour during storage in experiment of Molteberg *et al*. (1996). But there is some possible explanation of these results. The phenomenon of “reversion off-flavours” was described in oils containing higher level of linolenic fatty acid (Frankel, 1985). It is common characteristic of soybean oil (Min and Boff, 2002). Some initial products of

oxidation, especially unsaturated aldehydes, have very low thresholds. But during storage period, these unsaturated aldehydes can isomerise to more stable 2-alkenals with much higher threshold (Frankel, 1985).

Although lipids and unsaturated fatty acids are potential precursors for off-flavours, varieties ´Abel´ and ´Izak´, which differed most in sensory evaluation, had very similar lipid and unsaturated fatty acid content. That's why lipid content and total amount of unsaturated fatty acids could not be the only explanation of the varietal difference in sensory attribute. Similar results were obtained by Molteberg *et al*. (1996) in three Norway varieties ´Kapp´, ´Mustang´, and ´Svea´. Content of individual unsaturated fatty acids, which are oxidised in different rates (Molteberg *et al*., 1995; Visioli *et al*., 1998), also could not explain the varietal variation in sensory evaluation in this study. Different development of sensory changes could be related to the presence/absence of others compounds that were not analysed, especially other compounds contributing to flavour. Moletberg *et al*. (1996) investigated relationships between sensory attributes and phenolic compounds.

6. Conclusion

Lipid degradation, suspected to be the major cause of grain deterioration, was determined by increasing acidity due to increasing level of liberated fatty acids. Level of FFA were detected by direct titration according to Czech State Norm (CSN 56 0512-9). Storage stability of seven proso millet varieties in dependency on different processing designs and storing conditions was evaluated. Changes of sensory attributes in correlation to increasing FFA during long-term storage of whole grains were detected in three oat varieties.

Evaluated collection of six proso millet varieties demonstrated wide range of morphological and phenological characteristics. Remarkably short period to maturity of variety ´Irtyskoe 201´ indicated its suitability for planting in regions with short period of convenient conditions. Fat content was estimated in negative correlation to WTS.

Proso millet varieties were significantly higher in unsaturated fatty acids, especially in linoleic acid. Oat varieties were higher in total fat content. Level of unsaturated fatty acid influenced development of titratable acidity. Total fat content affected titratable acidity only in proso millet varieties, but not in oat varieties. It can be assumed the influence of other compounds related to development of rancidity, especially compounds with antioxidant activity, which can be investigated in further experiments.

Titratable acidity was significantly affected by processing designs and storage conditions. The most significant changes occurred in samples stored in room temperature conditions in laboratory and in processed grains, especially in those processed with hulls. Titratable acidity significantly increased in neither proso millet grains nor oat grains in both storing conditions.

Storing at lower temperature is more suitable and contributes to minimizing of storage changes. Among processing designs, grains were the most stable in both storing conditions. Grains can be recommended for storing in unsuitable conditions, which is very common problem in tropics and subtropics. Groats are not suitable for storing even in freezer; they should be prepared before consumption exactly. Varieties with lower content of unsaturated fatty acids and lower level of linoleic acid are more resistant to development of rancidity and can be recommended for storing of high-fat cereals.

Changes in sensory attributes did not follow changes of titratable acidity of oat varieties. Although titratable acidity did not change significantly, taste and bitterness render

grains inedible at the end of storage period. This inconsistency could be caused by minute threshold of produced compounds. Development of sensory changes was influenced by neither content of lipid nor content of unsaturated fatty acids. Compounds determining development of sensory changes should be the subject of further investigations.

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8. Appendices

Figure A1: Temperature and relative humidity in laboratory conditions**.**

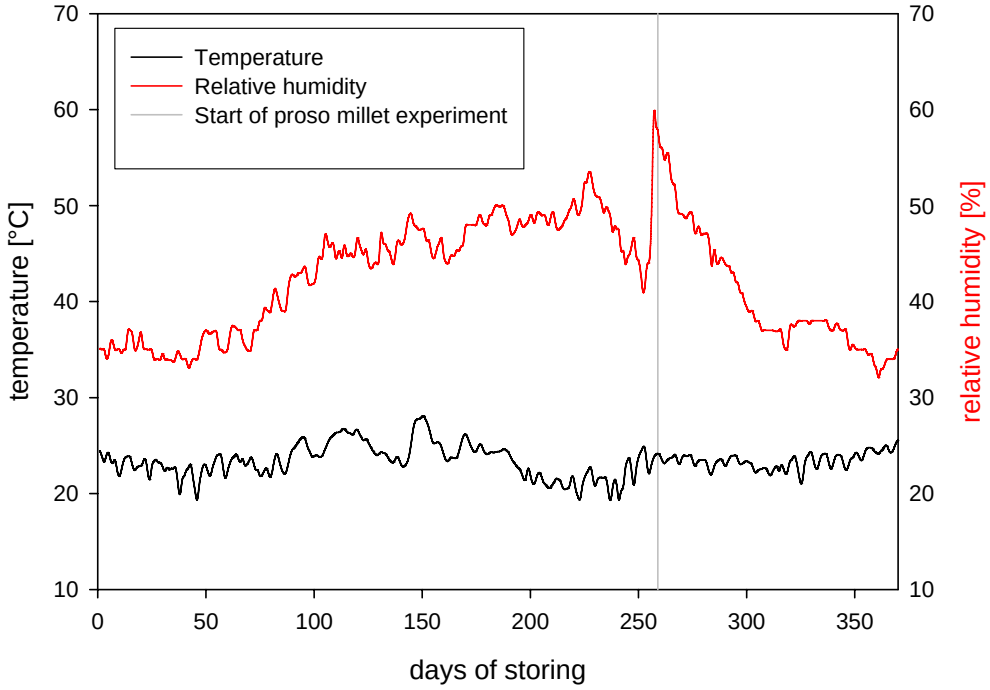

Figure A2: Format sheet for sensory evaluation

Surname: _____ Date:
Evaluated following sensory descriptors of given samples.

Odour

Sample no. very pleasant_____ disgusting

Sample no. very pleasant_____ disgusting

Sample no. very pleasant_____ disgusting

Taste

Sample no. delicious_____ disgusting

Sample no. delicious_____ disgusting

Sample no. delicious_____ disgusting

Intensity of bitterness

Sample no. without bitterness_____ bitterness

Sample no. without bitterness_____ bitterness

Sample no. without bitterness_____ bitterness

Figure A3: Average temperature during vegetation periods in monthly averages [°C].

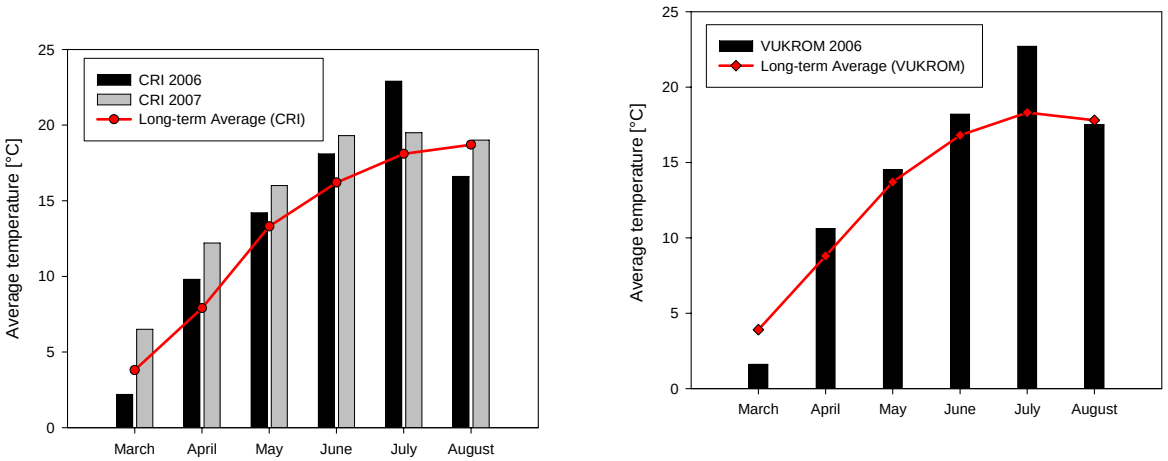

Figure A4: Average sums of precipitation during vegetation periods.

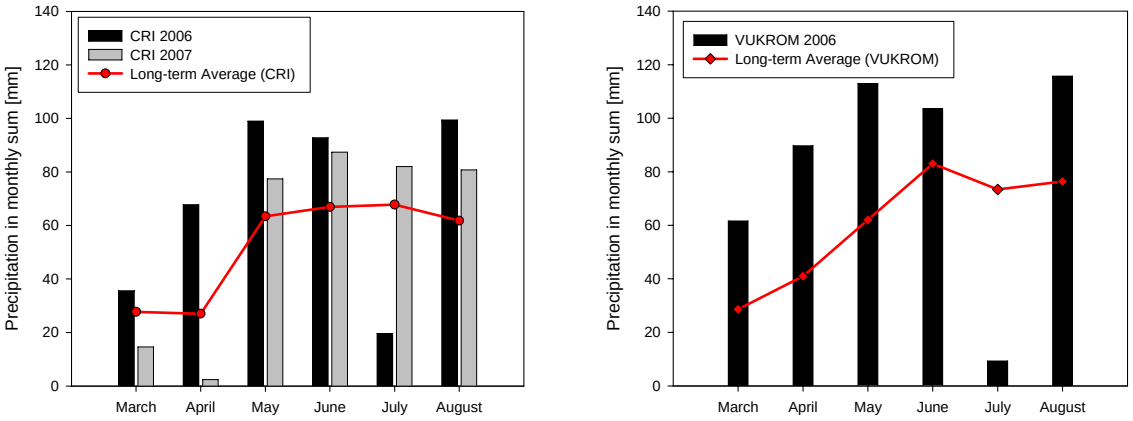

Table A1: Fatty acid distribution in oat and proso millet varieties (mean values ± SD).

Fatty acids [%]	Abbr.	Oats	Proso
Lauric	12:0	3.93 ± 2.99^{b}	1.01 ± 0.95^{bc}
Myristic	14:0	0.63 ± 0.07^{a}	0.15 ± 0.03^{a}
Palmitic	16:0	22.16 ± 0.91^{c}	10.07 ± 0.72^{d}
Palmitoleic	16:1	0.36 ± 0.07^{a}	0.22 ± 0.04^{a}
Stearic	18:0	1.37 ± 0.23^{ab}	1.50 ± 0.55^{c}
Oleic	18:1	34.06 ± 1.49^{d}	21.19 ± 0.31^{e}
Linoleic	18:2	37.96 ± 1.05^{e}	63.64 ± 0.83^{f}
Linolenic	18:3	1.16 ± 0.19^{ab}	1.06 ± 0.057^{c}
Arachic	20:0	0.08 ± 0.02^{a}	0.34 ± 0.08^{ab}
Gadoleic	20:1	0.54 ± 0.03^{a}	0.28 ± 0.03^{a}
Behenic	22:0	0.08 ± 0.03^{a}	0.31 ± 0.15^{ab}
Erucic	22:1	1.08 ± 0.13^{ab}	-
Lignoceric	24:0	0.06 ± 0.01^{a}	0.13 ± 0.05^{a}
Fat content		5.85 ± 0.65	4.19 ± 0.23

Values of parameters marked by same index are not significantly different at $p \leq 0.05$.

Table A2: Evaluation of morphological and phenolocial features of proso millet varieties (mean values ± SD).

	Mean ± SE (Standard Error)	**Range**	**CV** Coefficient of Variation
Days from emergence to flowering	26 ± 1	19-35	18.978
Days from emergence to maturity	66 ± 3	45-85	18.159
Plant height [cm]	92.1 ± 7.6	69.0-166.3	28.394
WTS [g]	6.82 ± 0.21	5.52-7.86	10.637
Yield [$kg.h^{-1}$]	2154 ± 237	1112-3444	38.191
Fat in dry matter [%]	4.36 ± 0.11	3.93-4.94	8.558

Table A3: Average titratable acidity of proso millet varieties (mean values ± SD)

		Flour	**Groats**	**Whole grain**
		[mg of NaOH/100g of dry matter]		
Period	week 0	42.7 ± 4.3^{f}	74.4 ± 9.4^{d}	48.8 ± 2.7^{a}
	week 2	49.1 ± 4.0^{a}	95.5 ± 13.1^{e}	50.0 ± 2.7^{b}
	week 4	51.4 ± 5.0^{ab}	105.6 ± 15.8^{f}	51.4 ± 2.8^{c}
	week 6	54.0 ± 5.4^{b}	112.9 ± 13.4^{g}	53.2 ± 2.8^{d}
	week 8	57.4 ± 5.3^{c}	120.5 ± 12.0^{h}	$54,7 \pm 3.1^{e}$
	week 10	59.1 ± 5.5^{c}	129.2 ± 11.6^{a}	56.1 ± 3.2^{f}
	week 12	61.9 ± 6.7^{d}	135.1 ± 11.2^{ab}	57.5 ± 3.4
	week 14	63.9 ± 7.1^{de}	140.7 ± 8.2^{bc}	58.7 ± 3.7^{h}
	week 16	65.1 ± 7.1^{d}	145.2 ± 8.6^{c}	60.2 ± 3.8^{i}

Values of parameters marked by same index are not significantly different at $p \leq 0.05$.

Table A4: Average titratable acidity of oat varieties **(**mean values ± SD).

	´Abel´	***´Izak´***	***´Saul´***
	[mg of NaOH/100g of dry matter]		
0 month	90.3 ± 3.5^{bcd}	80.6 ± 1.3^{b}	72.7 ± 1.9^{b}
1 month	71.63 ± 5.9^{a}	66.3 ± 3.0^{a}	52.2 ± 3.5^{a}
2 months	90.7 ± 3.5^{cd}	82.6 ± 5.9^{b}	72.4 ± 1.1^{b}
3 months	89.4 ± 3.7^{bcd}	81.9 ± 4.7^{b}	72.5 ± 1.7^{b}
4 months	89.9 ± 2.4^{bcd}	82.4 ± 4.9^{b}	72.7 ± 1.6^{b}
5 months	88.9 ± 2.0^{bcd}	80.6 ± 4.5^{b}	73.3 ± 1.5^{b}
6 months	88.7 ± 1.1^{bcd}	80.6 ± 4.9^{b}	72.5 ± 1.0^{b}
7 months	87.4 ± 1.2^{b}	81.2 ± 4.7^{b}	73.3 ± 1.1^{b}
8 months	87.8 ± 1.9^{bc}	81.3 ± 4.5^{b}	73.3 ± 1.3^{b}
9 months	88.3 ± 2.1^{bcd}	81.7 ± 4.9^{b}	73.6 ± 1.7^{b}
10 months	89.0 ± 2.9^{bcd}	81.9 ± 4.3^{b}	72.9 ± 1.8^{b}
11 months	91.1 ± 3.1^{d}	82.6 ± 4.7^{b}	73.5 ± 1.9^{b}
12 months	91.0 ± 3.1^{d}	83.0 ± 3.9^{b}	73.6 ± 1.8^{b}
Freezer	85.7 ± 5.8^{a}	76.7 ± 4.0^{a}	70.0 ± 6.2^{a}
Laboratory c.	90.5 ± 4.4^{b}	84.4 ± 4.8^{b}	72.8 ± 5.2^{b}
Average	88.1 ± 5.7	80.5 ± 5.9	71.4 ± 5.8

Values of parameters marked by same index are not significantly different at $p \leq 0.05$.